CALIFORNIA OLIVE COMMITTEE
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Clovis, CA 93611
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AGENDA
Ripe Olive Research Subcommittee Meeting
Double Tree • Sonoma Room
Thursday, November 30, 2017
12:00 p.m.
(Lunch to be provided at 11:30 a.m.)

I. Call to Order

   a. Roll call
   b. Election of Chairperson for Research Subcommittee (action item)
   c. Approval of 6-20-17 Research Subcommittee Minutes (action item)

II. Discussion and Review of 2017 Projects

   a. Budget Status Update

III. Presentation of 2018 Proposals

IV. Approval of 2018 Budget (action item)

   a. Closed Session

V. Approval of authority to the Executive Director and Chairman to approve No-Cost extensions. (action item)

VI. Approval of Authority for Inter-Item Transfers of the Research Budget (action item)

VII. Other Business

VIII. Adjournment

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COC Subcommittees for 2017-2019

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Mike Silveira, G-1
Mark Hendrixson, G-2
Dennis Burreson, MUS
Julia Tinsley, BCF
Tim T. Carter, BCF
Ed Curiel, G-1
Janet Edwards, MUS
Felix Musco, MUS
Edward Garcia, G-1
Mark Heuer, G-2
Pat Ricchiuti, G-2
Doug Reifsteck, BCF

Marketing Subcommittee:

Bill McFarland, MUS
Colleen Sparda, BCF
Tim T. Carter, BCF
Ed Curiel, G-1
Tracey Wood, MUS
Julia Inestroza, G-2
Pat Ricchiuti, G-2
Scott Hamilton, MUS
Mark Hendrixson, G-2
Phil Quigley, BCF
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Mike Silveira, G-1
Rick Benson, G-2
Pablo Nerey, G-1
Joan Whelan-Vanderhorst, G-2
Sergio Mendez, BCF
Vito DeLeonardis, G-2
Felix Musco, MUS

Inspection Subcommittee:

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Julia Inestroza, G-2
Dennis Burreson, MUS
Pablo Nerey, G-1
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Edward Garcia, G-1
Carolina Burreson, G-1
John Patterson, G-2
Galen Pfeiffer, G-2
Joan Whelan-Vanderhorst, G-2

Research Subcommittee:

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Julia Tinsley, BCF
Carolina Burreson, G-1
Mike Silveira, G-1
Bert Quezada, G-2
Vito DeLeonardis, G-2
Chris Henderson, G-1
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CALIFORNIA RIPE OLIVES
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CALIFORNIA OLIVE COMMITTEE

Research Subcommittee Meeting Minutes
Tuesday, June 20, 2017
11:00 a.m.
Double Tree- Modesto, CA
1150 9th Street

I. CALL TO ORDER

A meeting of the Research Subcommittee was called to order by Michael SILVEIRA at 10:27 a.m., and the following members were present:

Members	Affiliation:
Felix MUSCO	Musco
Bert QUEZADA	Grower
Ben HALL	Musco
Rick BENSON	Grower
Phil QUIGLEY	Bell-Carter
Mike SILVEIRA	Grower
Dennis BURRESON	Musco
Chris HENDERSON	Grower
Julia TINSLEY	Bell-Carter
Pat V. RICCHIUTI	Grower
Ed CURIEL	Grower
Vito DELEONARDIS	Grower
Pablo NEREY	Grower
Mark HEUER	Grower
Janet EDWARDS	MUSCO

Others Present:	
Alexander OTT	COC
Todd SANDERS	COC
Liza RAMON	COC
Elizabeth BROWN	COC
Jeff SMUTNY	USDA
Nathan O'Connor	USDA
Beth HEUER	GROWER
Rachelle BROSS	GROWER
Giulio ZAVOLTA	GROWER

With a majority of the Subcommittee members present, a quorum was established.

MOVED BY Julia TINSLEY, duly seconded by Chris HENDERSON, and unanimously carried THAT the minutes of the November 10, 2016 Research Subcommittee meeting be approved. (Motion 6.20.17 #1)

II. DISCUSSION AND APPROVAL OF 2018 PRIORITIES

Each year the Research Subcommittee sets priorities of research they would like executed on their behalf for the following year. These efforts are to fund more specific and calculated research to enhance the benefits to the industry. Once the priorities are set they are provided to the University of California liaisons to request proposals from researchers. Proposals will be reviewed for funding in November by the subcommittee.

List of Priorities:
PGR's and pruning treatments to manage alternate bearing
Epidemiology and management of olive knot
Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard
Canopy Management, Tree Hedging and topping to Optimize Yield
Northern Fly Trapping
Southern Fly Trapping
Understanding Styrene formation in olives
Full Nutrition analysis in green and black olives
Preliminary Field study to identify new olive fly control materials
DNA Olive
Acrylamide
Mechanical Harvesting

MOVED BY Pat RICCHIUTI, duly seconded by Vito DELEONARDIS, and unanimously carried THAT the existing projects with three new projects be approved for 2018 priorities. (Motion 6.20.17 #2)

VII. ADJOURNMENT

Chairman Dennis BURRESON adjourned the meeting at 11:19 a.m.

I hereby certify that the above is full, true and correct copy of the minutes of the meeting held on June 20, 2017 in Modesto, California, by the Subcommittee.

June 22, 2017 Liza Ramon

Date: June 22, 2017 Liza Ramon, California Olive Committee

SUMMARY OF MOTIONS FOR JUNE 20, 2017

Motion 6.20.17 #1 **APPROVED**

MOVED BY Julia TINSLEY, duly seconded by Chris HENDERSON, and unanimously carried THAT the minutes of the November 10, 2016 Research Subcommittee meeting be approved.

Motion 6.20.17 #2 **APPROVED**

MOVED BY Pat RICCHIUTI, duly seconded by Vito DELEONARDIS, and unanimously carried THAT the existing projects with three new projects be approved for 2018 priorities.

*****FOR YOUR INFORMATION*****

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: PROGRESS REPORTS for 2017

BACKGROUND: Each year, the Subcommittee funds research projects and request progress reports from researchers. Provided in your packet are the current research project progress reports.

2017 Research Projects

Updated 11/7/2017

Researcher	Project	Amount	Finalized MOU	Paid thus far	% Paid
Ferguson & Ficthner	Investigating Anti-Oxidant to Decrease the Leaf Abscission with Ethephon Applications	$ 39,996.00	1/30/17revised 3/7/2017	$ 7,999.20	20%
Wang	Investigation of chemical and biological formation of styrene in black ripe table olives	$ 51,350.00	2/17/2017	$29,450.00	57%
Wang	Comprehensive nutritional analysis of California green and black ripe table olives	$ 46,350.00	2/17/2017	$9,450.00	20%
Preece & Ferguson	Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard	$ 35,442.00	3/1/2017	$ 21,265.20	60%
Adaskaveg	Epidemiology and management of olive knot caused by Pseudomonas savastanoi pv. Savastanoi	$ 18,900.00	1/30/2017	$11,340.00	60%
Lovatt & Fichtner	Managing Alternate Bearing in olive with PGRs and Pruning	$ 23,845.00	2/17/2017	$14,307.00	60%
Rosecrance & Kruegar	Canopy Management, Tree Hedging and topping to Optimize Yield	$ 31,075.00	1/17/2017	$18,645.00	60%
Lightle	Prelimiary field study to identify new olive fly control materials	$ 19,647.00	4/7/2017	$11,788.20	60%
	Contingency	$ 100,000.00		$12,000.00	12%
Ernie Simpson	Northern Fly Trapping	$ 6,500.00	2/15/2017	$ 5,700.00	88%
Jim Stewart	Southern Fly Trapping	$ 6,333.33	2/17/2017	$5,541.69	88%
	Total	**$ 379,438.33**			0%

CALIFORNIA OLIVE COMMITTEE
PROJECT PROGRESS REPORT: 2017 SEASON

Workgroup/Department: Olive / Plant Sciences, UC Davis

Project Year 2017 Anticipated Duration of Project: 1 year

Project Title:
Investigating Anti-Oxidant Amendments to Decrease the Leaf Abscission with Ethephon Applications:

Project Leaders:
Dr. Louise Ferguson: Extension Specialist, Department of Plant Sciences, 2037 Wickson Hall, Mail Stop II, UC Davis, 1 Shields Ave., Davis CA 95616, (530) 752-0507 [Office], (559) 737-3061 [Cell], LFerguson@ucdavis.edu.

Dr. Elizabeth J. Ficthner: Farm Advisor, University of California Cooperative Extension, 4437 South Laspina Street, Tulare CA 93274. (559) 684-3310 (Office), (559) 684-2057 (Cell). EJFichtner@ucdavis.edu.

Cooperators:
Dr. Richard Rosecrance: Professor, Chico State University
Mr. William H. Krueger: Farm Advisor Emeritus
Mr. Erick Nielsen: ENE Inc., pruning and harvesting designer, fabricator and contractor.

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2017 Current Funding Request: **$39,996.00**

Problems and Significance:
Multiple studies, including our most recent California study (Burns et. al. 2008) have demonstrated that the higher concentrations of Ethephon required to decrease olive fruit removal force to make mechanical harvesting with trunk shakers more efficient also result in unacceptable levels of leaf abscission.

On October 11th 2016 a presentation at the International Society for Horticultural Science 8th International Olive Symposium in Split, Croatia a research group from Israel presented their results examining the anatomical and molecular differences between fruit and leaf abscission in table olives. The following is from their abstract discussion:

"We found that the the typical anatomical characteristics of the abscission zones such as small cells with less pectin compared to the neighboring cells, exist in the leaf but not the fruit abscission zone. Screening the response of the cultivars in our olive germplasm collection reveals differences in the response of the abscission zones of the leaves and fruits as expressed in their anatomical characteristics. Transcriptomic analysis of the of the various abscission zones

reveals induction of several hormones as well as cell wall degradation enzymes in the leaf and fruit abscission zones in response to exogenous ethylene. However, cellulase activation was found only in the leaf abscission zone. In addition, we found that reactive oxygen species mediated abscission in response to exogenous ethylene applications only in leaves. Thus, adding an antioxidant such as ascorbic or butyric acid to the abscission compound enhanced abscission of fruit but not leaves. Our findings suggest that advising growers to use an abscission agent exclusively tailored to induce the abscission of fruit would greatly promote the mechanized harvest of table olives". (Goldental-Cohen et. al. 2016)

The major table cultivar in Israel is Manzanilla so they have tested their theory on our major cultivar. The specific treatment they suggested was 0.3% ascorbic acid or 100 mM butyric acid added to the standard Ethephon treatment. As our cooperators Rosecrance and Krueger are currently conducting a mechanical pruning and harvesting experiment in California we arranged to have a preliminary trial done this October 15th 2016. Hopefully we will have the results for proposal review in 2016.

In fall of 2017 we proposed to evaluate the ability of both 0.3% ascorbic acid in combination with ethephon to enhance fruit removal efficiency without producing unacceptable leaf abscission when using trunk shaking mechanical to harvest Manzanillo olives. Drs. Louise Ferguson, Elizabeth Fichtner and Richard Rosecrance and Farm Advisor Emeritus William H. Krueger MSc will be the cooperators.

Progress through 10/15/2016:

A preliminary application of 0.3 ascorbic acid was applied by Dr. Richard Rosecrance and William H. Krueger, Farm Advisor Emeritus in the Nickles Estate moderate density olive block October 15th 2016. Effect on fruit pull force was evaluated on October 25th; there was no significant drop in fruit pull force.

2017 Objective: (April 1st – December 31st 2017)

Evaluate the ability of the best suggested treatment:

a. 0.3% ascorbic acid

to decrease fruit removal force and increase harvest efficiency of a trunk shaking harvester without producing more than 25% leaf loss.

2017 Experimental Procedures Completed:

Orchards pruned for trunk shaker harvesting was secured:

1. Nickles moderate density orchard (203 trees/acre) in Colusa County

Experimental design was be a randomized complete block: within 9 rows of each treatment was assigned once: 4 treatments x 3 trees x 10 replications (rows) = 120 treated trees: See Att. I

Sept. 29th 2017 9 randomly selected sets of 3 trees/treatment were sprayed to drip with the following treatments at the 100 GPA rate:

1. 2000 PPM Ethephon and 0.25% surfactant
2. 2000 PPM Ethephon and 0.25% surfactant + 0.3% ascorbic acid

3. 0.3% ascorbic acid and 0.25% surfactant
4. a water control and 0.25% surfactant

Before Harvest:
Fruit detachment force was taken from the middle tree of each 3 tree set on 10 shoots per tree with at least 5 olives per shoot before application and at 7 day intervals until harvest.

At Harvest:
At harvest the middle tree of the three was be harvested by trunk shaker, then hand gleaned.

Both sets of fruit were be weighed and samples submitted to Musco Olive for sample grades and value.

We did not to submit this set of fruit samples for canning, sensory and consumer evaluation unless the COC Research Subcommittee wants these tests done. We prefer to determine if the technique works before investigating effects on processed fruit quality. Also, Ethephon is unregistered.

After Harvest:
The middle tree of the treated tree sets will be evaluated monthly for leaf drop at harvest through the beginning of shoot growth the following spring:

1. The trees were visually rated for leaf drop on a 1-3 scale: 1= none, 2 = visible, 3 = severe.
2. Ten shoots per tree will be counted for % leaf drop: > 25% will be considered unacceptable.

Data was analyzed using ANOVA with an LSD means separation.

Desired Result:
The 2000 ppm ethephon treatment will decrease fruit removal force, increase harvesting efficiency to at least 90% without producing leaf loss over 25%.

First Analyzed Results: Att. II
As the attached results show when sprayed with the water control treatment and water control treatment + 3% ascorbic acid ~ 72% of the olives were removed by the trunk shaker. Adding Ethephon® to the spray increased fruit removal by ~ 6-8%, to 78-80%. However, neither Ethephon® treatment significantly decreased the pull force.

And, as the attached results show the Ethephon® alone and with 0.3% ascorbic acid significantly increased leaf loss as of harvest October 23rd. On a scale of 0-3, with three being the highest; the Ethephon® treated trees were rated at 1.5 (Ethephon® alone) and 2.5 (Ethephon® + 0.3% ascorbic acid); both significant levels of leaf loss versus the water control.

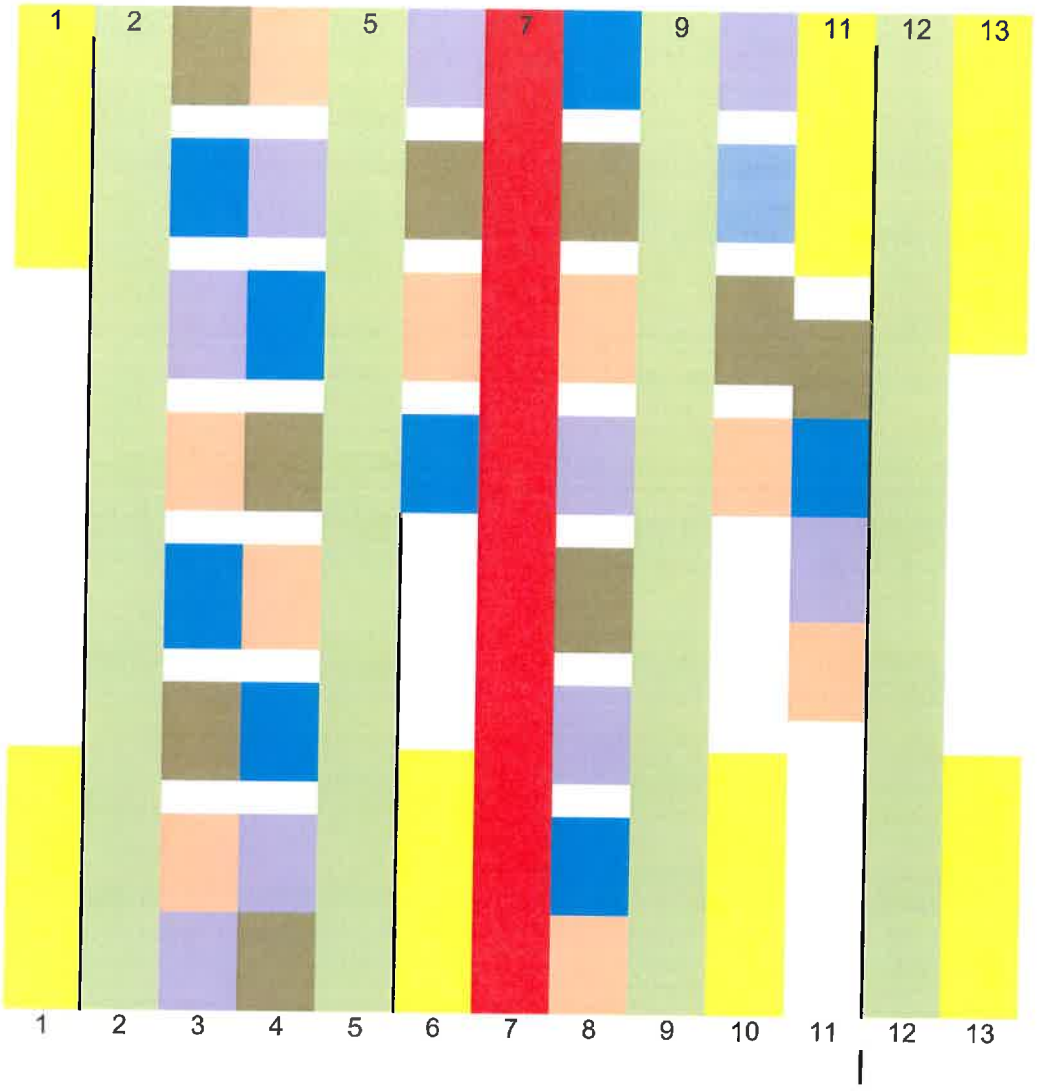
The final report will follow leaf loss through March 2018. However, the analyzed data demonstrates that while Ethephon® produced a modest 6-8 % increase in fruit harvest efficiency, and a modest decrease in pull force, from 0.5 kg for the control treatment to 0.4 kg for the Ethephon® treated trees, the leaf loss for Ethephon® treated trees at harvest was significant. On a

scale of 1-3, with 3 being the most severe leaf loss, the water control treatments, with and without 0.3% ascorbic acid, had leaf drop ratings below 0.5 while Ethephon® treated trees, with and without 0.3% ascorbic acid had leaf drop ratings of 1.5 and 2.2 respectively. These results demonstrate adding 0.3% ascorbic acid to Ethephon® did not significantly decrease fruit pull force, harvest efficiency or leaf loss

References:

Burns, J.K., L. Ferguson, K. Glozer, W.H. Krueger, and R.C., Rosecrance. 2008. Screening fruit loosening agents for black ripe processed table olives. HortScience 43(5):1449-1453.

Goldental-Cohen, S, I.B.Y. Mani, B. Avidan, S. Lavee, G. Ben-Ari. 2016. Anatomical and molecular differences between the olive fruit and leaf abscission zone enable development of a selective abscission compound. *Abstract*: Int. Soc. Of Hort. Sci.: 8th Int. Olive Symp. Oct. 10th – 14th 2016 Split, Croatia. P. 42.

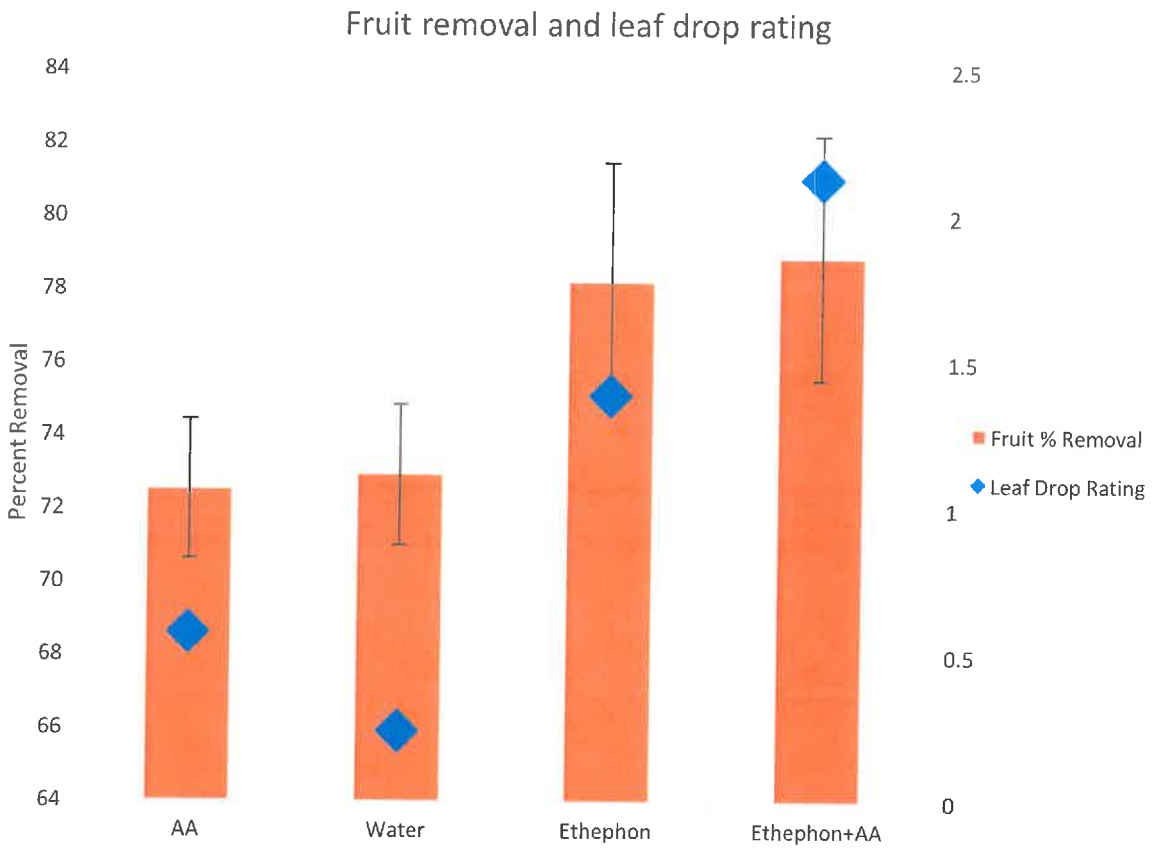


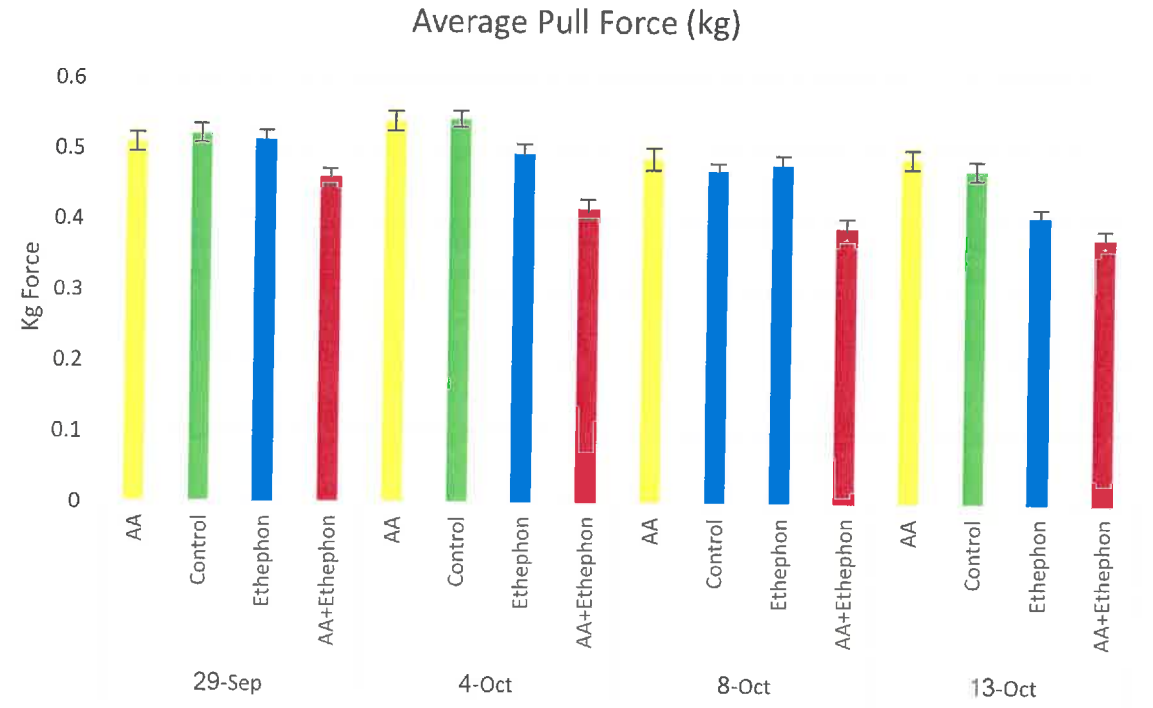
Note: Planted 7-8-01. Tree spacing =12'x18' or 202 trees/ac
S = Sevillano (pollinators) center row budded to Sevillano 07-03
The rest of the trees are Manzanillos
Plot is located on Greenbay Avenue (Nickels Estate in Arbuckle).

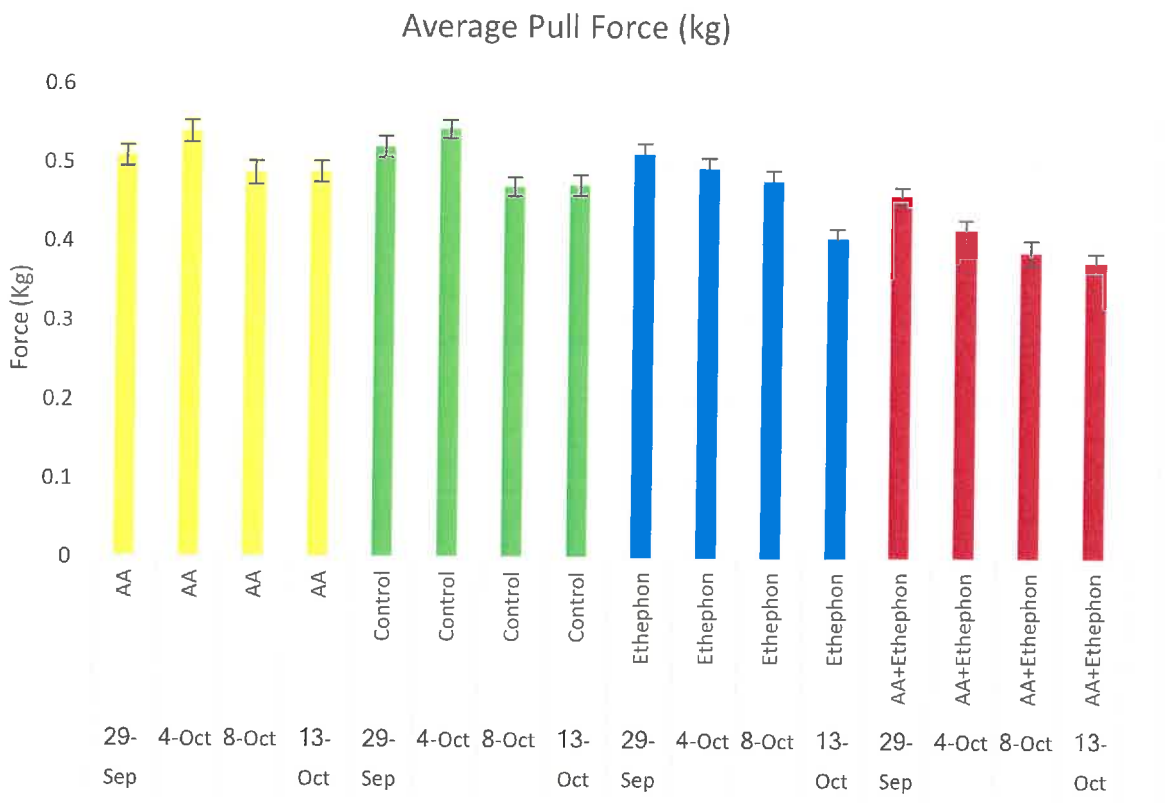
N

g Treatment5/25-27/16

10 foot topping followed by hand pruning to remove stubs with thinning cu
13 foot topping followed by hand pruning to thin canopy and remove stubs
Hand Pruned with thinning cuts


Fruit removal and leaf drop rating
Percent Removal
84
82
80
78
76
74
72
70
68
66
64
2.5
2
1.5
1
0.5
0
AA
Water
Ethephon
Ethephon+AA
Fruit % Removal
Leaf Drop Rating


Average Pull Force (kg)
Kg Force
0.6
0.5
0.4
0.3
0.2
0.1
0
AA
Control
Ethephon
AA+Ethephon
29-Sep
4-Oct
8-Oct
13-Oct


Average Pull Force (kg)
Force (Kg)
0.6
0.5
0.4
0.3
0.2
0.1
0
AA
AA
AA
AA
Control
Control
Control
Control
Ethephon
Ethephon
Ethephon
Ethephon
AA+Ethephon
AA+Ethephon
AA+Ethephon
AA+Ethephon
29-Sep
4-Oct
8-Oct
13-Oct
29-Sep
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29-Sep
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8-Oct
13-Oct
29-Sep
4-Oct
8-Oct
13-Oct

Investigating the formation of styrene in California-style ripe table olives

Objective

The goal of this project is to understand the mechanism of styrene contamination and/or formation in the black ripe olive production process. Specific objectives include:

1. Compare levels of styrene in domestic and imported California-style black and green ripe olives.
2. Isolate and identify microbiota from these olives to determine species that are abundant in high-styrene olives.
3. Investigate the formation of styrene *in vitro* by incubating these isolated microbes with precursor compounds.
4. Inoculate olives with metabolically active microbes and quantify styrene formation under various storage conditions

Materials

Samples: Forty samples of California-style olives were provided by the producers or purchased from online retailers or grocery stores.

Reagents: Styrene and styrene d8 were purchased from Sigma Aldrich (St. Louis, MO).

Methods

Analysis of styrene in California-style olives. Thirty grams of olive was blended with 50 mL nanopure water. An additional 100 mL water was used to rinse the blender and 54 g baked sodium chloride was added to olive slurry. An aliquot of the mixture (60 mL) was transferred to a small amber bottle sealed with a septum cap. The SPME fiber (DVB/CAR/PDMS) was inserted into the headspace of the bottle for 30 min to extract styrene. The fiber was then manually injected into the GC-MS for analysis. Analyses were conducted in triplicate.

Results

- Domestic samples contained significantly less styrene than imported samples (Table 1).
- Seven out of twelve imported black ripe samples had higher than 0.3 µg/g styrene (Figure 1). Styrene has an odor threshold of 0.32 µg/g, meaning that sensory defects may be detectable in these samples.
- With the exception of one sample (0.2 µg/g), all other domestic olives had less than 0.055 µg/g styrene (Figure 1).

		Styrene concentration (µg/g)
Domestic	**Black (n=20)**	0.021 ± 0.044
	Green (n=6)	nd*
Imported	**Black (n=12)**	1.37 ± 1.83
	Green (n=2)	0.051 ± 0.044

Table 1. Average styrene concentration of domestic and imported black and green ripe olives

*nd = below detection limit

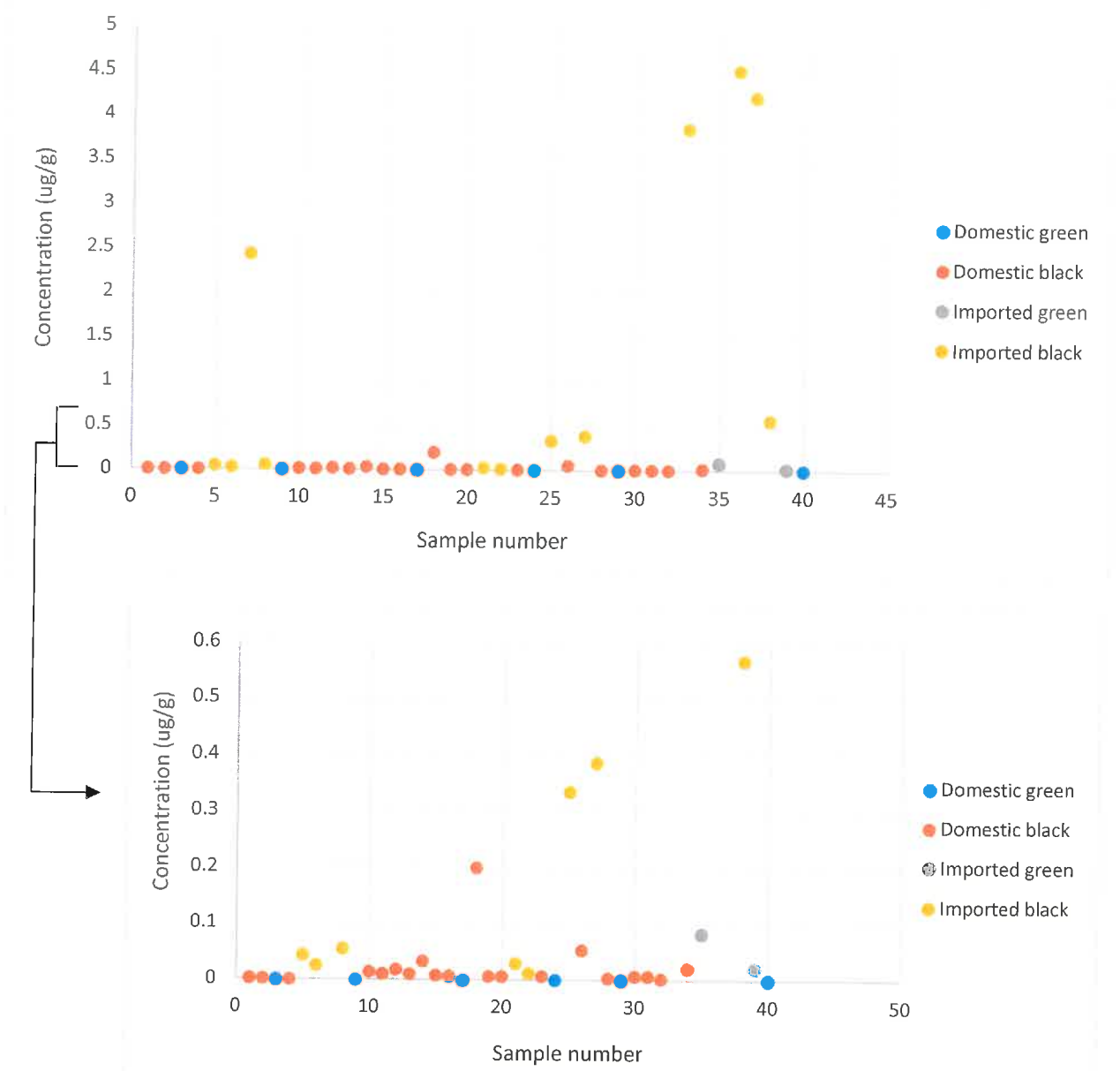

Figure 1. Styrene concentration in domestic and imported California-style olives

- Domestic green ripe olives did not have any detectable styrene and imported green ripe olives had significantly less styrene than imported black ripe samples.
- Samples 2, 30 and 32 were domestic samples processed without ferrous gluconate. There was no clear difference between these olives and traditional black ripe olives, which suggests that styrene content is not influenced by ferrous gluconate treatment.
- All domestic green ripe olives were processed fresh, whereas all domestic black ripe samples in this study were stored before processing. These results support the hypothesis that microbial growth during olive storage is causing production of styrene.
- No identifiable trend exists between olive style (whole, sliced, chopped), fruit size or cultivar and styrene content.

Future work

Isolate and identify of microbiota from high-styrene olives Oct-Nov. 2017

Olive tissue will be macerated in PBS and frozen at -20°C. DNA will be extracted using MoBio PowerFood microbial kit. PCR amplification will be performed on the V4 region of 16s rRNA genes for bacteria and the internal transcribed spacer (ITS) region for yeasts. DNA will be analyzed using Illumina sequencing at the UC Davis Genome Center. A FASTQ file containing reads will be subjected to bioinformatics analysis.

Assess styrene production by isolated microbes in vitro Dec-Jan. 2017

Microorganisms identified in high-styrene olives will be obtained from the UC Davis Phaff Yeast Culture and/or other commercial producers. Microbes will be individually inoculated into buffered peptone water fortified with cinnamic, p-coumaric, caffeic and ferulic acid (phenolic precursors to styrene and styrene derivatives). Following 3 days of incubation, the concentration of phenolic compounds and styrene/derivatives will be measured. Microorganisms that demonstrate the ability to convert phenolic precursors into styrene will be considered metabolically active.

Inoculate olives with metabolically active microbes to assess styrene production in vivo Jan-Feb. 2017

Fresh olives with negligible styrene content will be placed into brine. These olives will receive four different treatments in triplicate: (1) inoculation with metabolically active microorganisms only; (2) inoculation with all abundant microorganisms from the high-styrene olives; (3) no inoculation; (4) no inoculation + nisin/natamycin (to inhibit bacterial/yeast growth). The olives will be incubated and the phenolic/volatile profile will be measured every 3 days for 2 weeks, and then every 7 days for an additional 2 weeks. The microbiota of olives from each treatment will be analyzed.

Appendix

Table S1: Characteristics and styrene concentrations of California-style olive samples

Sample #	Color	Style	Size	Cultivar*	Origin	Styrene (µg/g)
1	Black	Sliced		Manzanilla	Domestic	0.0022 ± 0.0005
2	Black	Whole	Medium	Manzanilla	Domestic	0.0016 ± 0.0005
3	Green	Whole	Medium	Manzanilla	Domestic	0.0000
4	Black	Whole	Extra large	Manzanilla	Domestic	0.0000
5	Black	Whole	Jumbo	Gordal	Imported	0.0430 ± 0.014
6	Black	Whole	Large		Imported	0.0251 ± 0.0020
7	Black	Whole	Large		Imported	2.43 ± 0.072
8	Black	Whole	Large		Imported	0.0546 ± 0.0074
9	Green	Whole	Large	Manzanilla	Domestic	0.0000
10	Black	Whole	Jumbo	Sevillano	Domestic	0.0141 ± 0.0036
11	Black	Whole	Collosal	Sevillano	Domestic	0.0099 ± 0.0024
12	Black	Whole	Large	Manzanilla	Domestic	0.0181 ± 0.0059
13	Black	Chopped		Manzanilla	Domestic	0.0100 ± 0.0019
14	Black	Whole	Medium	Manzanilla	Domestic	0.0331 ± 0.0017
15	Black	Whole	Small	Manzanilla	Domestic	0.0081 ± 0.0008
16	Black	Whole	Extra large	Manzanilla	Domestic	0.0070 ± 0.0037
17	Green	Whole	Medium	Manzanilla	Domestic	0.0000
18	Black	Sliced		Mission	Domestic	0.1994 ± 0.013
19	Black	Whole	Jumbo	Barouni	Domestic	0.0067 ± 0.0025
20	Black	Chopped			Domestic	0.0063 ± 0.0002
21	Black	Whole	Large		Imported	0.0296 ± 0.003
22	Black	Whole	Extra large		Imported	0.0122 ± 0.0007
23	Black	Broken		Sevillano	Domestic	0.0070 ± 0.0013
24	Green	Whole	Medium	Manzanilla	Domestic	0.0000
25	Black	Whole	Large		Imported	0.3351 ± 0.026
26	Black	Sliced			Domestic	0.0531 ± 0.034
27	Black	Whole	Large		Imported	0.3862 ± 0.036
28	Black	Sliced			Domestic	0.0035 ± 0.0029
29	Green	Whole	Medium	Manzanilla	Domestic	0.0000
30	Black	Whole	Medium	Manzanilla	Domestic	0.0072 ± 0.0017
31	Black	Whole	Large		Domestic	0.0069 ± 0.001
32	Black	Whole	Medium	Manzanilla	Domestic	0.0026 ± 0.0007
33	Black	Whole	Medium		Imported	3.8528 ± 0.42
34	Black	Whole	Jumbo	Barouni	Domestic	0.0211 ± 0.0099
35	Green	Whole		Manzanilla	Imported	0.0821 ± 0.0032
36	Black	Whole	Extra large		Imported	4.5124 ± 0.60
37	Black	Whole	Medium		Imported	4.2105 ± 0.70
38	Black	Whole	Medium		Imported	0.5684 ± 0.056
39	Green	Whole		Manzanilla	Imported	0.0200 ± 0.0032
40	Green	Whole	Medium	Manzanilla	Domestic	0.0000

*Cultivars were unknown for many imported samples and samples purchased at the grocery store

Nutritional analysis of California-style black and green ripe table olives

Objective

The goal of this project was to comprehensively measure the nutritional and chemical profile of domestic and imported black and green ripe olives in order to a) identify any potential health benefits or risks in table olives and b) to determine effects of processing method, sample origin and cultivar on chemical composition.

Materials

Samples: Twelve samples were provided by Bell Carter. Ten samples were provided by Musco. The remaining eighteen samples were purchased from local grocery stores (Davis, CA) or online from Walmart. Sample characteristics are summarized Table S1 (appendix).

Reagents. HPLC grade methanol, dimethyl sulfoxide (DMSO), acetic acid and hexane were purchased from Fisher Scientific (Fairlawn, NJ). Hydroxytyrosol, tyrosol, p-coumaric acid, caffeic acid, benzoic acid, acrylamide, acrylamide d3 and alpha-tocopherol standards were purchased from Sigma Aldrich (St. Louis, MO).

Methods

Fifty grams of olives were removed from the can, dried with a paper towel and homogenized in a food processor. A summary of analyses using this pulp is displayed in Figure 1.

Individual phenolics: Olive pulp (2 g) was vortexed with 10 mL dimethyl sulfoxide (DMSO) for 1 min and centrifuged (9000 rpm, 5 min). The extract was filtered (0.45 μm, nylon) and 0.25 mL was diluted with 0.25 mL methanol and 0.5 mL water. Samples were stored at -20°C until analysis using ultra performance liquid chromatography coupled to a diode array detector (UPLC-DAD).

Total phenols: DMSO extract (0.1 mL) from the individual phenolics assay was diluted with 1.9 mL water. Folin Ciocalteau reagent (0.1 mL) was added and the sample was briefly vortexed. One mL sodium carbonate (200 g/L) was added and the sample was vortexed again and placed in the dark for 45 min. Absorbance was measured at 725 nm using a spectrophotometer.

Acrylamide: Olive pulp (2 g) was shaken with 6 mL methanol:water (50:50, v/v) for 2 min. Two mL hexane was added and the sample was shaken again for 2 min to remove lipid interferences. The sample was centrifuged (8000 rpm, 5 min), filtered using vacuum filtration and stored at -20°C. Prior to analysis, four mL extract was evaporated at 60°C and the residue was redissolved in 1 mL water. The extract was filtered (0.45 μm, nylon) and analyzed using UPLC-DAD.

Benzoic acid: Olive pulp (2 g) was vortexed with 10 mL methanol for 1 min and centrifuged (8000 rpm, 5 min). Extract was filtered (0.45 μm, nylon), diluted 1 to 2 with water and stored at -20°C until analysis with UPLC-DAD.

Oil extraction: Olive pulp (5 g) was shaken with 25 mL hexane for 2 min. The mixture was centrifuged (5000 rpm, 5 min) and the hexane layer was evaporated in order to isolate the extracted oil.

Tocopherols: Oil (40 µl) was dissolved in 160 µL of hexane. Ethanol (200 µL) and methanol (600 µL) were added to the sample, which was vortexed for 1 min and centrifuged (5000 rpm, 5 min). Samples were stored at -20°C to allow oil to fully separate from the organic phase. The extract was filtered (0.45 µm, nylon) and analyzed using UPLC-DAD.

Fatty acid profile: Oil (10 µL) was dissolved in 4 mL toluene. The sample was mixed with 3 mL methanol plus 0.6 mL methanol/HCl (80:20, v/v) and heated at 80°C for 1 hour. Hexane (1.5 mL) and nanopure water (1 mL) were added to the extract, which was briefly vortexed. The sample sat for 5 minutes to allow separation of phases and the upper phase containing fatty acid methyl esters (FAMEs) was passed over anhydrous sodium sulfate to remove any additional water. Solutions were analyzed using gas chromatography flame ionization detection (GC-FID).

Ferrous gluconate: Ferrous gluconate was measured as a function of iron content. Olive pulp was frozen at -80°C and submitted to the UC Davis Analytical Lab for analysis. Nitric acid/hydrogen peroxide microwave digestion was used, followed by quantitation with inductively coupled plasma atomic emission spectrometry (ICP-AES).

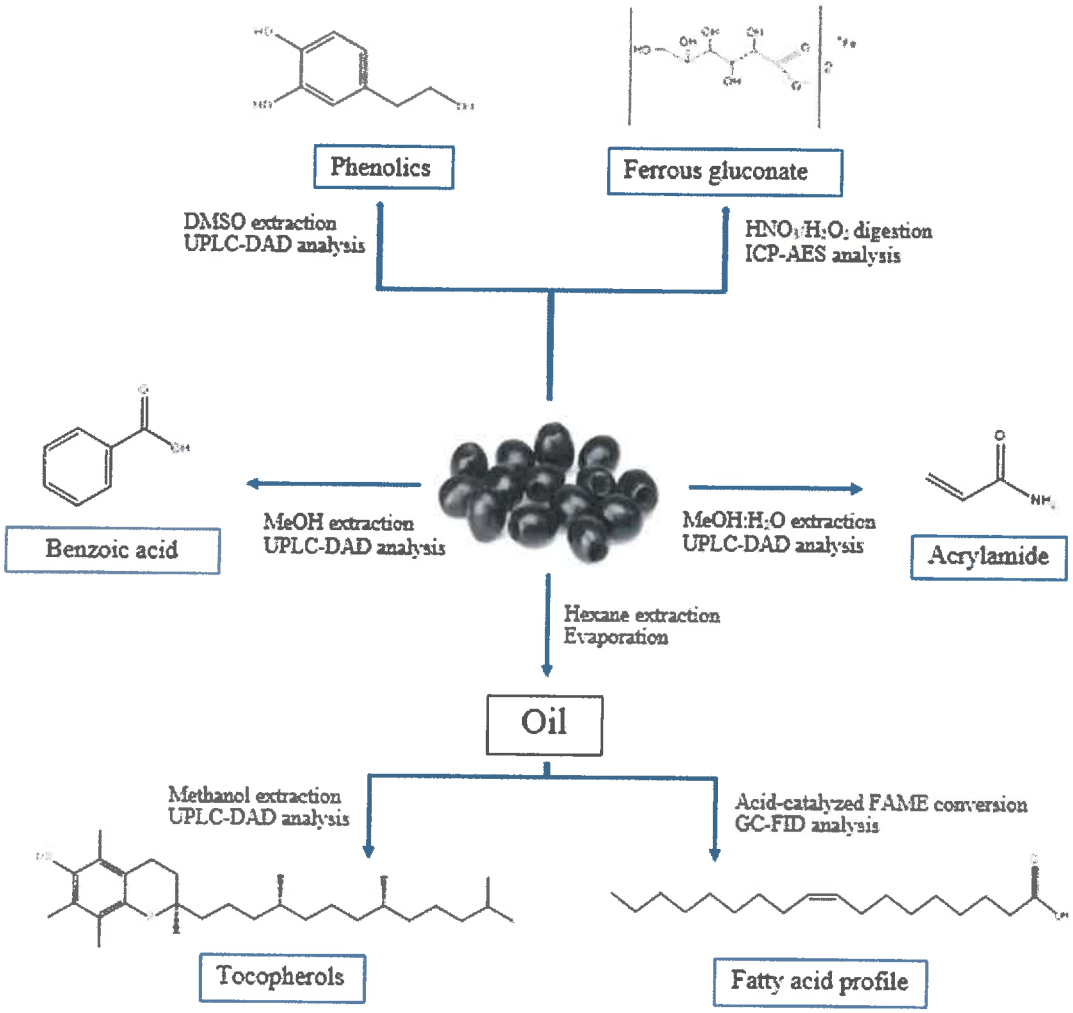

Figure 1. Summary of extraction and analytical methods

Results

Table S2 in the appendix contains data for individual samples.

Beneficial compounds (phenolics/tocopherols):

- Imported green ripe olives had significantly higher phenolics than the three other categories of olives (Figure 2). However, only two imported green ripe samples (both from the same brand) were analyzed.
- The variability in phenolics is very high when all domestic olives are considered together. However, separating the data by processor greatly reduced variability. In the case of one processor, green ripe olives had significantly higher phenolics than black ripe (see individual reports).
- Figure 3 shows that individual phenolic profile may be cultivar-dependent, although more samples of varying cultivars are needed to support this conclusion.
- There was no significant difference in α-tocopherol content based on processing method, even when data was separated by processor, or sample origin (Figure 2). β-tocopherol and γ-tocopherol were not identified in any sample.

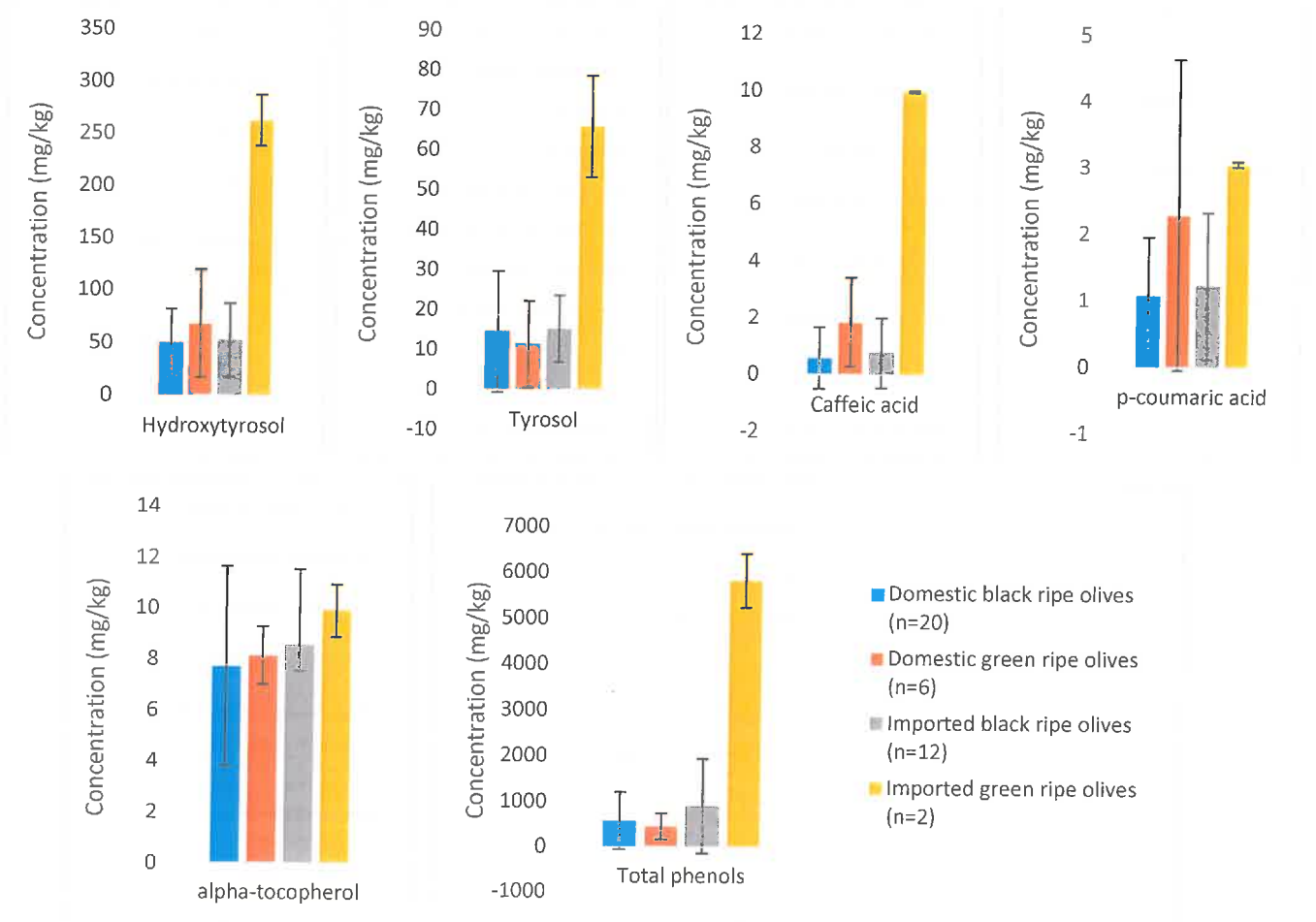

Figure 2. Phenolic compound and α-tocopherol content of domestic and imported black and green ripe olives.

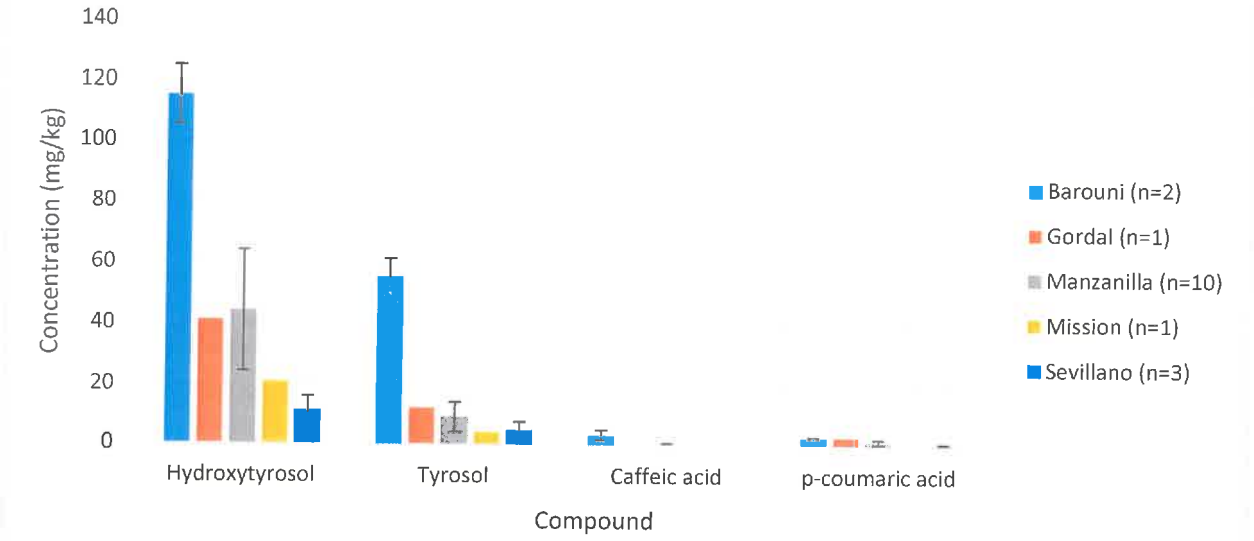

Figure 3. Phenolic composition of black ripe olives separated by cultivar.

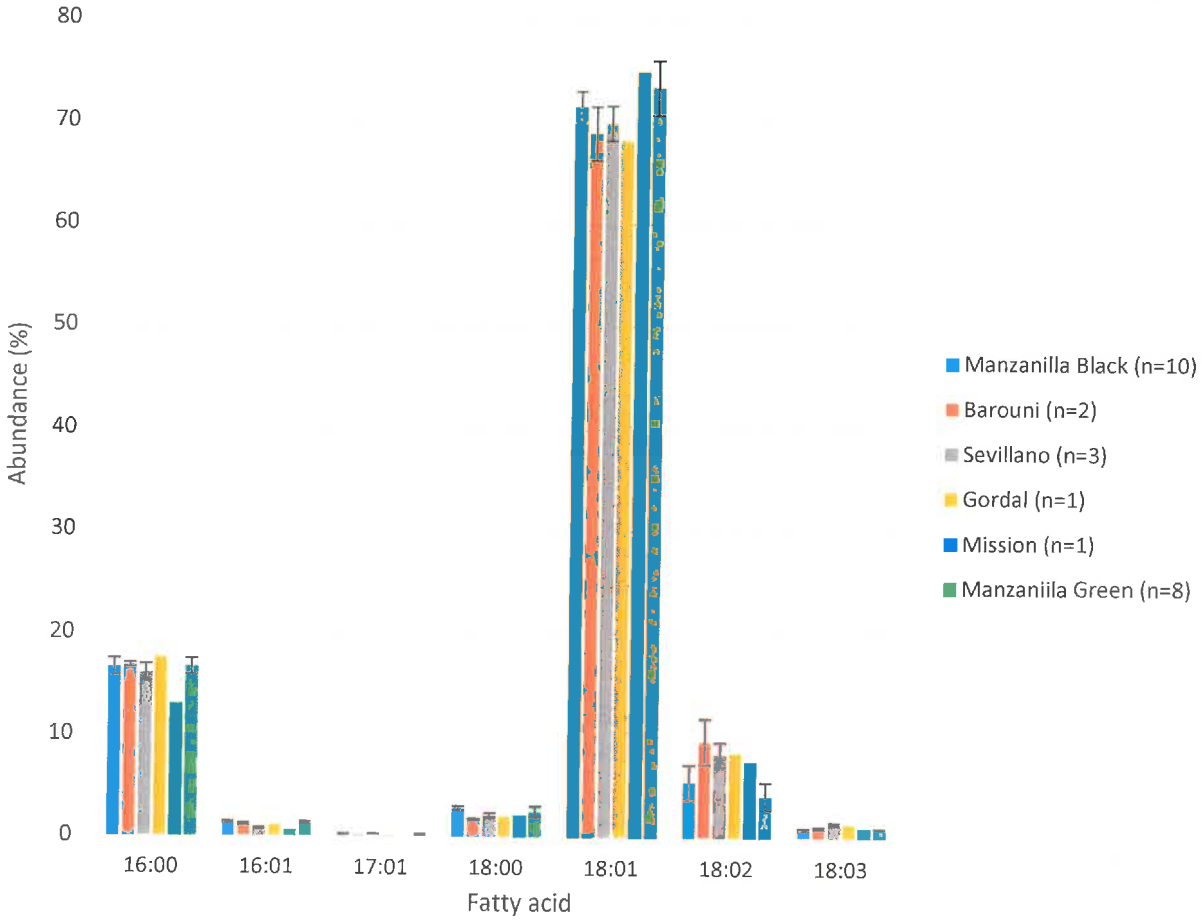

Figure 4. Fatty acid profile of black and green ripe olives separated by cultivar.
*Fatty acids 17:0, 20:0, 22:0 and 24:0 are not displayed because percentages were very low and consistent among all samples.

		16:0	16:1	17:1	18:0	18:1	18:2	18:3
Domestic	**Black (n=20)**	16.6 ± 1.4	1.32 ± 0.3	0.23 ± 0.08	2.59 ± 0.4	70.9 ± 2.1	6.21 ± 2.1	1.07 ± 0.2
	Green (n=6)	16.9 ± 0.5	1.41 ±0.09	0.25 ± 0.03	2.80 ± 0.2	72.1 ± 0.7	4.53 ± 1.0	0.94 ± 0.09
Imported	**Black (n=12)**	15.2 ± 1.4	0.96 ± 0.3	0.23 ± 0.06	2.37 ± 0.4	72.4 ± 2.0	6.81 ± 0.9	1.04 ± 0.2
	Green (n=2)	15.6 ± 0.4	1.20 ± 0.04	0.08 ± 0.007	1.80 ± 0.003	77.1 ± 0.5	2.47 ± 0.04	0.91 ± 0.03

Table 1. Comparison of fatty acid profile based on sample origin and processing method.
*Fatty acids 17:0, 20:0, 22:0 and 24:0 are not displayed because percentages were very low and consistent among all samples

Fatty acid profile:

- As expected, oleic acid (18:1 n9) was the most abundant fatty acid in all olive samples (Table 1).
- There was no significant difference in fatty acid profile between domestic black and green ripe olives, even when controlling for cultivar
- The results disprove our initial hypothesis that eliminating air bubbling during processing would decrease oxidation and increase the content of oleic acid and polyunsaturated fatty acids, 18:2 and 18:3.
- Domestic black olives are not significantly different from imported black olives. However, imported green olives had a unique 17:1, 18:0, 18:1 and 18:2 profile.
- Figure 4 shows the subtle variation in fatty acid profile based on olive cultivar. If a sufficient number of samples are analyzed, it may be possible to use multivariate statistics to cluster cultivars based on fatty acid ratios.

Processing residues (benzoic acid/ferrous gluconate):

- Domestic green ripe olives contained less benzoic acid than domestic black ripe olives (Table 2).
- For domestic green ripe olives, the variability in benzoic acid decreases when processors are considered separately (see individual reports).
- For black ripe olives, the processor, olive style do not appear to influence benzoic acid concentrations.
- Interestingly, the three samples with the highest benzoic acid concentrations were all "jumbo" sized olives (two Barouni, one Gordal). However, there was otherwise no identifiable trend between cultivar or fruit size and benzoic acid.
- FDA regulations (CFR 21) set a maximum level of 0.1% in foods, or 1000 mg/kg. All samples were far below this limit.
- Sorbic acid was not identified in any samples.

		Benzoic acid (mg/kg)
Domestic	**Black (n=20)**	18.4 ± 17.2
	Green (n=6)	2.04 ± 2.45
Imported	**Black (n=12)**	6.83 ± 12.4
	Green (n=2)	8.78 ± 0.019

Table 2. Benzoic acid concentrations in domestic and imported olives

- Ferrous gluconate….

Acrylamide:

- _____Fill in!_____

Conclusions

- Green ripe olives contained higher phenolics than black ripe olives in some cases, possibly due to elimination of air bubbling and reduced oxidation during processing.
- Characteristics of oil in the fruit (α-tocopherol content and fatty acid profile) did not seem to be affected by processing method.
- Phenolic content and fatty acid profile influenced by olive cultivar.
- Domestic green ripe olives contained less benzoic acid than black ripe. However, all samples were well below FDA regulations.

Appendix

Table S1: Characteristics of California-style olive samples

Sample #	Color	Style	Size	Cultivar*	Origin
1	Black	Sliced		Manzanilla	Domestic
2	Black	Whole	Medium	Manzanilla	Domestic
3	Green	Whole	Medium	Manzanilla	Domestic
4	Black	Whole	Extra large	Manzanilla	Domestic
5	Black	Whole	Jumbo	Gordal	Imported
6	Black	Whole	Large		Imported
7	Black	Whole	Large		Imported
8	Black	Whole	Large		Imported
9	Green	Whole	Large	Manzanilla	Domestic
10	Black	Whole	Jumbo	Sevillano	Domestic
11	Black	Whole	Collosal	Sevillano	Domestic
12	Black	Whole	Large	Manzanilla	Domestic
13	Black	Chopped		Manzanilla	Domestic
14	Black	Whole	Medium	Manzanilla	Domestic
15	Black	Whole	Small	Manzanilla	Domestic
16	Black	Whole	Extra large	Manzanilla	Domestic
17	Green	Whole	Medium	Manzanilla	Domestic
18	Black	Sliced		Mission	Domestic
19	Black	Whole	Jumbo	Barouni	Domestic
20	Black	Chopped			Domestic
21	Black	Whole	Large		Imported
22	Black	Whole	Extra large		Imported
23	Black	Broken		Sevillano	Domestic
24	Green	Whole	Medium	Manzanilla	Domestic
25	Black	Whole	Large		Imported
26	Black	Sliced			Domestic
27	Black	Whole	Large		Imported
28	Black	Sliced			Domestic
29	Green	Whole	Medium	Manzanilla	Domestic
30	Black	Whole	Medium	Manzanilla	Domestic
31	Black	Whole	Large		Domestic
32	Black	Whole	Medium	Manzanilla	Domestic
33	Black	Whole	Medium		Imported
34	Black	Whole	Jumbo	Barouni	Domestic
35	Green	Whole		Manzanilla	Imported
36	Black	Whole	Extra large		Imported
37	Black	Whole	Medium		Imported
38	Black	Whole	Medium		Imported
39	Green	Whole		Manzanilla	Imported
40	Green	Whole	Medium	Manzanilla	Domestic

Table S2: Phenolic, benzoic acid, acrylamide and ferrous gluconate content of forty samples (mg/kg)

Sample #	Hydroxytyrosol	Tyrosol	Caffeic acid	p-coumaric acid	Total phenols	α-tocopherol	Benzoic acid	Acrylamide	Ferrous gluconate
1	68.2 ± 0.9	9.5 ± 1.3	0.2 ± 0.01	0.6 ± 0.01	358 ± 36.4	8.4 ± 0.6	18.6 ± 0.24	±	±
2	66.1 ± 3.0	7.7 ± 1.7	1.0 ± 0.1	1.7 ± 0.10	342 ± 17.6	7.2 ± 0.3	0.0	±	±
3	112.4 ± 19.5	16.9 ± 2.5	3.4 ± 0.7	4.6 ± 0.83	669 ± 55.4	10.1 ± 1.1	5.2 ± 0.52	±	±
4	56.2 ± 3.3	11.7 ± 1.6	0.3 ± 0.04	1.6 ± 0.12	270 ± 19.1	7.0 ± 1.8	16.3 ± 0.04	±	±
5*	41.1 ± 1.2	13.3 ± 0.4	0.3 ± 0.03	2.8 ± 0.14	234 ± 8.0	±	43.4 ± 0.10	±	±
6	36.7 ± 1.5	15.0 ± 0.7	0.3 ± 0.04	0.0	395 ± 24.9	10.1 ± 0.9	0.8 ± 0.30	±	±
7	71.9 ± 4.7	16.4 ± 1.1	1.2 ± 0.1	1.6 ± 0.11	409 ± 52.4	8.9 ± 0.5	2.9 ± 0.11	±	±
8	26.9 ± 1.0	9.7 ± 1.0	0.4 ± 0.03	1.1 ± 0.06	223 ± 2.4	8.5 ± 1.7	0.0 ± 0.00	±	±
9	23.1 ± 0.7	2.4 ± 0.2	0.7 ± 0.03	0.5 ± 0.03	139 ± 25.4	6.8 ± 1.1	1.0 ± 0.04	±	±
10	9.6 ± 0.9	4.4 ± 0.2	0.0	0.5 ± 0.01	125 ± 13.8	5.0 ± 0.3	18.4 ± 0.01	±	±
11	7.2 ± 0.3	2.7 ± 0.2	0.0	0.1 ± 0.001	103 ± 0.7	5.2 ± 0.3	11.0 ± 0.96	±	±
12	29.4 ± 2.1	4.5 ± 1.2	0.2 ± 0.04	1.0 ± 0.09	221 ± 30.2	7.0 ± 0.70	17.2 ± 1.50	±	±
13	42.3 ± 2.0	9.2 ± 0.2	0.0	0.2 ± 0.001	244 ± 51.9	3.7 ± 0.2	1.6 ± 0.02	±	±
14	50.9 ± 1.3	9.7 ± 0.1	0.3 ± 0.01	1.3 ± 0.04	330 ± 3.3	9.5 ± 1.3	38.1 ± 0.58	±	±
15	9.1 ± 0.2	1.1 ± 0.1	0.0	0.4 ± 0.03	105 ± 6.4	7.2 ± 0.9	0.0	±	±
16	17.1 ± 2.6	6.2 ± 0.2	0.2 ± 0.1	0.5 ± 0.11	203 ± 51.2	4.5 ± 0.4	7.6 ± 1.36	±	±
17	75.7 ± 2.2	8.9 ± 0.6	2.0 ± 0.04	2.5 ± 0.10	390 ± 21.9	8.3 ± 0.4	2.2 ± 0.04	±	±
18	20.7 ± 2.5	4.2 ± 1.0	0.0	0.0	130 ± 29.2	5.8 ± 0.04	1.8 ± 0.002	±	±
19	122.1 ± 4.1	59.3 ± 1.4	4.5 ± 0.2	2.8 ± 0.16	836 ± 144.1	15.5 ± 0.9	61.8 ± 0.16	±	±
20*	83.8 ± 2.3	15.7 ± 0.3	0.2 ± 0.03	0.5 ± 0.05	412 ± 17.5	±	11.2 ± 0.26	±	±
21	17.9 ± 1.4	3.1 ± 0.5	0.0	0.0 ±	224 ± 136.8	6.3 ± 0.4	0.9 ± 0.07	±	±
22	6.6 ± 0.1	4.5 ± 0.1	0.0	0.3 ± 0.02	63 ± 20.9	1.6 ± 0.4	16.6 ± 0.30	±	±
23*	16.6 ± 0.7	7.8 ± 0.5	0.0	0.6 ± 0.07	102 ± 17.3	0.81 ±	22.9 ± 0.77	±	±
24	20.7 ± 0.5	3.1 ± 0.0	0.2 ± 0.02	0.0 ±	108 ± 13.3	8.2 ± 0.2	0.0	±	±
25	64.7 ± 2.4	21.6 ± 0.6	0.7 ± 0.1	0.8 ± 0.07	493 ± 12.0	9.5 ± 0.5	1.8 ± 0.23	±	±
26	50.5 ± 0.8	15.8 ± 0.1	0.4 ± 0.03	1.0 ± 0.08	330 ± 3.3	12.7 ± 0.2	17.1 ± 0.01	±	±
27	22.2 ± 1.0	5.8 ± 0.2	0.0	0.4 ± 0.04	162 ± 32.0	7.1 ± 1.0	9.0 ± 0.17	±	±
28	66.0 ± 2.2	13.5 ± 1.4	0.4 ± 0.02	0.8 ± 0.02	326 ± 2.1	10.8 ± 1.0	15.0 ± 0.26	±	±
29	143.6 ± 11.7	30.8 ± 2.3	4.0 ± 0.3	5.6 ± 0.34	693 ± 155.3	8.2 ± 1.2	4.8 ± 0.50	±	±

30	57.4	±	0.3	12.1	±	0.5	0.7	±	0.02	1.4	±	0.01	1389	±	128.1	7.0	±	0.6	1.2	±	0.08	±	±
31	63.1	±	1.2	23.5	±	1.1	0.0			1.0	±	0.08	1564	±	41.5	7.2	±	0.2	21.7	±	1.12	±	±
32	43.9	±	1.5	19.2	±	0.7	0.8	±	0.04	3.1	±	0.18	1518	±	80.1	5.7	±	0.3	32.1	±	1.68	±	±
33	68.6	±	2.1	19.0	±	0.5	0.7	±	0.03	0.8	±	0.06	1862	±	110.2	13.2	±	0.7	2.1	±	0.20	±	±
34	108.4	±	0.8	50.9	±	0.9	2.2	±	0.01	2.4	±	0.07	2329	±	285.4	16.6	±	0.9	55.4	±	0.02	±	±
35	245.8	±	35.8	57.1	±	5.7	9.9	±	1.4	3.1	±	0.50	5433	±	308.1	9.2	±	0.5	8.8	±	2.35	±	±
36	58.3	±	1.3	19.3	±	0.4	0.7	±	0.1	1.4	±	0.09	1516	±	147.4	9.2	±	0.1	0.6	±	0.26	±	±
37	133.6	±	1.2	30.6	±	0.3	4.5	±	0.1	3.6	±	0.08	3486	±	527.0	11.0	±	0.07	2.1	±	0.10	±	±
38	76.4	±	6.1	24.3	±	0.7	0.3	±	0.1	1.7	±	0.07	1633	±	15.4	9.0	±	1.1	1.9	±	0.10	±	±
39	279.1	±	5.3	75.1	±	1.4	10.0	±	0.2	3.0	±	0.04	6259	±	369.3	10.7	±	1.3	8.8	±	0.47	±	±
40	32.9	±	0.1	6.3	±	0.9	0.8	±	0.03	0.6	±	0.02	694	±	57.7	7.3	±	1.0	0.0			±	±

*Insufficient oil was extracted from these samples, limiting α-tocopherol analysis

Table S3: Fatty acid profiles of the 40 California-style olive samples (expressed as %)

Sample #	16:0	16:1	17:0	17:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	24:0
1	16.5	1.3	0.2	0.3	2.8	73.0	3.9	0.9	0.5	0.3	0.1	0.2
2	16.4	1.5	0.2	0.3	2.8	72.2	5.0	0.8	0.5	0.1	0.1	0.1
3	17.3	1.4	0.2	0.2	2.5	72.8	3.6	1.1	0.4	0.3	0.1	0.1
4	16.6	1.3	0.2	0.2	3.0	71.1	5.9	0.8	0.5	0.3	0.1	0.1
5	17.5	1.1	0.2	0.2	2.1	68.2	8.4	1.4	0.4	0.3	0.1	0.1
6	14.6	0.8	0.1	0.2	2.2	73.8	6.5	0.9	0.4	0.3	0.1	0.1
7	13.6	0.7	0.2	0.2	2.8	73.1	7.8	0.8	0.4	0.3	0.1	0.1
8	14.9	0.8	0.1	0.2	2.2	73.2	6.9	1.0	0.4	0.3	0.1	0.1
9	16.8	1.5	0.2	0.2	3.1	71.4	5.0	0.9	0.5	0.3	0.1	0.1
10	16.5	0.9	0.2	0.3	2.3	67.9	9.2	1.6	0.4	0.3	0.1	0.1
11	15.1	0.9	0.2	0.3	2.0	71.0	8.3	1.3	0.4	0.3	0.1	0.1
12	15.7	1.4	0.2	0.3	2.9	70.4	7.3	0.9	0.5	0.3	0.1	0.1
13	16.8	1.4	0.2	0.2	2.9	71.3	4.9	1.2	0.5	0.3	0.1	0.1
14	18.0	1.7	0.1	0.2	3.0	71.1	3.9	1.1	0.5	0.3	0.1	0.1
15	15.3	1.4	0.2	0.3	2.3	73.7	5.0	1.0	0.4	0.3	0.1	0.1
16	15.9	1.5	0.2	0.2	3.1	68.1	9.2	0.8	0.5	0.2	0.1	0.1
17	16.4	1.3	0.2	0.3	2.7	72.7	4.4	0.9	0.5	0.3	0.2	0.2
18	12.9	0.7	0	0.1	2.2	74.9	7.5	1.0	0.3	0.3	0.1	0.1
19	16.8	1.3	0	0.1	1.8	67.0	11.0	1.1	0.3	0.3	0.1	0.1
20	19.0	1.7	0.2	0.2	2.8	68.0	5.8	1.3	0.5	0.3	0.1	0.1
21	17.1	1.4	0.2	0.3	2.6	71.6	5.2	0.9	0.4	0.3	0.1	0.1
22	15.4	0.8	0.2	0.4	2.1	72.5	6.5	1.3	0.4	0.3	0.1	0.1
23	16.4	0.8	0.2	0.3	2.3	70.8	6.8	1.5	0.4	0.3	0.1	0.1
24	17.1	1.5	0.1	0.2	2.7	71.3	5.3	0.9	0.4	0.2	0.1	0.1
25	13.7	0.8	0.1	0.2	2.4	74.7	6.3	0.9	0.3	0.3	0.1	0.1
26	20.1	1.7	0.1	0.2	2.7	69.1	3.8	1.3	0.5	0.3	0.1	0.1
27	16.3	1.6	0.1	0.2	3.3	70.3	6.2	1.0	0.5	0.2	0.1	0.1
28	16.4	1.3	0.1	0.3	2.9	73.0	4.0	1.1	0.4	0.3	0.1	0.1
29	17.6	1.4	0.2	0.2	3.0	72.6	3.3	0.9	0.4	0.2	0.1	0.1
30	16.8	1.3	0.2	0.3	3.0	72.6	3.3	0.9	0.4	0.2	0.1	0
31	16.9	1.6	0.1	0.2	2.6	71.3	5.4	1.0	0.4	0.3	0.1	0.1
32	16.7	1.4	0.2	0.3	3.0	71.5	5.2	0.9	0.4	0.2	0.1	0.1
33	15.6	1.0	0.1	0.2	2.1	71.4	7.7	1.1	0.3	0.3	0.1	0.1
34	16.6	1.2	0	0.1	1.7	70.7	7.9	1.0	0.4	0.3	0.1	0.1
35	15.9	1.2	0	0.1	1.8	76.8	2.5	0.9	0.3	0.3	0.1	0.1
36	14.6	0.8	0.1	0.2	2.1	72.7	7.5	1.1	0.3	0.3	0.1	0.1
37	16.2	1.0	0.1	0.2	2.1	71.3	6.9	1.2	0.4	0.3	0.1	0.1
38	13.4	0.7	0.2	0.3	2.5	75.5	5.8	0.8	0.4	0.3	0.1	0.1
39	15.3	1.2	0	0.1	1.8	77.5	2.4	0.9	0.3	0.3	0.1	0.1
40	16.5	1.4	0.2	0.3	2.7	71.5	5.7	0.9	0.4	0.3	0.1	0.1

CALIFORNIA OLIVE COMMITTEE

PROJECT 2017 YEAR INTERIM PROGRESS REPORT

Workgroup/Department: Olive / Plant Sciences, UC Davis

Project Year 2017- 2018 Anticipated Duration of Project: 10 years

Project Title:
Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard

Project Leaders:
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Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2013 Current Funding Request: **35,442.00**

Problems and Significance:
To facilitate mechanical harvesting the newest table olive orchards are planted in hedgerows and require regular mechanical pruning to keep the trees small. Our 12 X 18' foot research planting established at Nickels Soils Laboratory in 2002 has demonstrated to us this will be difficult with the 'Manzanillo' olive cultivar. Such hedgerow 'Manzanillo' orchards designed for mechanical harvesting would be easier to maintain if they could be grafted on dwarfing rootstocks. Among those olives with promise for use as a dwarfing rootstocks are:
Nikitskaya,
Olea cuspidate
Verticillium Resistant Oblonga
Dwarf D
Little Ollie (2015 addition)

In 2013 we proposed propagating these rootstocks and testing with grafted and non-grafted own rooted 'Manzanillo' controls for their dwarfing potential with 'Manzanillo' to produce a tree that is more amenable to mechanical harvesting. The own rooted 'Manzanillos' and 'Manzanillo' grafted to 'Manzanillo' in this orchard could also serve as the next generation hedgerow trained mechanically pruned orchard for mechanical harvesting with trunk and canopy contact shakers.

In 2013 year we were awarded funding to propagate the desired rootstocks and locate a suitable orchard site for establishment of the propagated trees. Both objectives have been achieved but due to difficulty of propagation with some cultivars and difficulty in locating a site with proper infrastructure planting was in spring 2014.

Overall Progress through 6/27/2017:

This application for initial funding was for two purposes:

I. **Propagation and grafting of the rootstocks with 'Manzanillo' scions.**
   a. Dr. John Preece supervised the development of specific propagation techniques for 112 each of the following olive cultivars to be used as dwarfing rootstocks; Nikitskaya, *Olea cuspidate,* Verticillium Resistant Oblonga and Dwarf D. Dwarf D proved very difficult to root as cuttings and this means that there were sufficient trees only for the closer spacing. At the wider spacing, Little Ollie, which roots easily is being tested, which adds another potential rootstock and expands the scope of the study in a logical way.

II. **Establishing the next generation olive hedgerow orchard for evaluation of mechanical harvesters.**
   a. Field 3556, a four-acre block located in Plant Sciences Field Facility located on the UC Davis Campus and maintained by UC Davis Plant Sciences field personnel was chosen as the planting site. This site has the added advantage of being located adjacent to oil orchards being developed by the UC Olive Center. The trees were planted in 2014. Attachment I: Field Map: 3556.

III. **Experimental Field Design:**
   a. Split plot design with the north half of the field at spaced at 10 X 16' and the south at 10 X 8'.
   b. There are 4 Randomized Complete Blocks
   c. Four different dwarfing rootstocks grafted with 'Manzanillo'
   d. Own rooted 'Manzanillo' and 'Manzanillo' grafted to a 'Manzanillo' grafting controls.
   e. Sevillano pollinizers were planted as border rows around the perimeter of the orchard and in the middle, as a row between the wide and narrow spacing.

2016-17 Objectives:

I. **Finish grafting all rootstocks, once the 2015 plants are established: Attachment I: Field 3556 Plot Map**

II. **Collect data to study the any growth differences among the scions on the different rootstocks compared to the controls; will be done end of September**

Experimental Procedures: 2015-2016;
Complete grafting of smallest rootstocks. Based on experience gained in grafting, the final trees planted in 2015 will be sufficiently large for grafting late summer, 2016. This will be completed and will add Little Ollie as an experimental rootstock at the wider spacing.

Two scions were bark or whip grafted onto each rootstock. During 2016, the weaker of the two grafts will be pruned off to a single scion per rootstock.

The goal is to be able to dwarf the olive trees by using one or more of these rootstocks. Therefore, data will focus on measurements of vegetative vigor, including branch numbers and lengths, tree height, tree caliper of both the rootstock and scion. During 2015, there were fruit on the Manzanillo, and although it is early in the study yield data will be collected. In 2016.

Data will be analyzed using ANOVA with an LSD means separation.

Progress Summary: 2015-2016
The trees planted in 2014 were maintained and staked and grown through the summer of 2015 to allow the trees to reach sufficient size for grafting. The 'Oblonga' trees were falling over more and in more need of staking (which was done) than the others. In spring of 2015, the border rows of 'Sevillano' pollinizers were completed by planting the last 41 trees. There were insufficient trees available in 2014 to complete the border rows.

Some of the rows of dwarf olives were incomplete, therefore additional cuttings were rooted and trees produced at the National Clonal Germplasm Repository nursery. The exception is that 'Dwarf D' has proven to be extremely difficult to root to produce plants for the wider spacing portion of the study. Therefore, in addition, cuttings of 'Little Ollie' were rooted and this cultivar proved to be easy to propagate. On September 29 2015 the nursery produced plants were planted into the orchard and 'Little Ollie' replaced the originally planned 'Dwarf D' at the wider spacing. This completes the planting and also gives a fifth genetically different rootstock to test for dwarfing of olive. One of the 'Sevillano' trees died during the summer of 2015, but there were a few extra trees from the spring 2015 planting, and that tree was replaced.
Sierra Gold Nursery and staff of the National Clonal Germplasm Repository grafted the trees from September 28 – Oct. 1, 2015. This cooler time of the year was better for the grafts to heal and take. Following grafting, the orchard was sprayed with Kocide to control olive knot.

The block was pruned May 15-18, 206. The block was rated July 20th 2016 with the following results: of the grafts done in September 28th 23 (3%) failed, and 87 rootstocks (11%) remain too small to graft, and 48 (6%) of the trees are dead or missing: Attachment I. The 3% graft failures and 11% too small in FALL 2015 will be grafted fall 2016. The 11% dead is due to squirrel damage to the irrigation lines flooding individual trees. The lines have been repaired and moved further away from the trees as they are now larger; in winter 2016 the drippers will be replaced with microsprinklers.

A few trees have produced minimal crop in 2016 so yield will be collected in September 2016.

By spring 2017 most of the trees should be large enough to demonstrate if the rootstocks have dwarfing potential and all the scions will be pruned back to an equal size to allow the Manzanillo scions to grow.

Progress Summary: 2016-2017

The dwarfing olive planting was pruned on May 25 and May 30, 2017. This pruning included the grafted trees and the border rows so that the trees in the guard rows will not overgrow the grafted trees. The grafted trees were pruned to a nurse limb and the graft scions and the non--ungrafted border trees were pruned in a similar manner. Following pruning, the trees were sprayed with copper. Any grafts that failed were marked for regrafting this season. Growth and yield data will be collected in the November, 2017.

Figure 1. Grafted olives pruned to one nurse limb: May 25th 2017.

Figure 2. Border row of 'Sevillano' trees after pruning: May 25th 2017.

Desired Result:

At maturity the rootstocks will maintain tree size at 10 feet or less, and the trees can be harvested with trunk shakers or canopy contact harvesters. The experimental design will also allow a determination of 'Manzanillo' tree yields at a 10 X 16' and an 8 X 16' feet spacing.

Department of Plant Pathology
Relevant AES/CE Project No.: 11103864

University of California
Division of Agricultural Sciences
INTERIM PROJECT/RESEARCH PROGRESS REPORT
California Olive Committee/California Olive Oil Commission
October 2017

Project Year: 2017

Project Leader:
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Title: Epidemiology and management of olive knot caused by *Pseudomonas savastanoi* pv. *savastanoi (Psv)*

2017 Research Objectives:

1) **Develop novel chemicals to improve performance of copper-based bactericides against *Psv***
   a) In-vitro sensitivity of *Psv* to copper in the presence of SBH (and potential derivatives) using selected copper/SBH ratios.
   b) Efficacy of copper-SBH mixtures for the management of olive knot caused by copper-sensitive and -resistant strains of *Psv* in field studies.
      i) Evaluate selected copper/SBH ratios with the goal to minimize the amount of copper applied while maintaining good disease control.

2) **Evaluate a biopesticide and several food additives for the control of olive knot**
   a) Determine the efficacy of the bio-pesticide Serenade (*Bacillus subtilis* strain QST 713) in field studies for the management of olive knot.
   b) Determine the efficacy of the GRAS food additives nisin, epsilon-poly-L-lysine, and lactic acid in field studies for the management of olive knot.

Summary of Progress in 2017 including ongoing studies:

1a. In-vitro sensitivity of* Psv *to copper in the presence of SBH (and potential derivatives). In-vitro studies were done using the spiral gradient endpoint method. Eight Cu-sensitive (growing at ≤25 mg/L MCE) or -resistant (growing at ≥50 mg/L MCE) *Psv* strains exposed to a SBH concentration gradient from 0.3 to 31 mg/L in absence of presence of 10, 25, or 50 mg/L metallic copper equivalent (MCE). SBH by itself was not inhibitory at ≤31 mg/L to any of the strains. When the SBH gradient was combined with copper at 10 mg/L MCE, inhibition was observed for all strains. The range of minimal inhibitory concentrations (concentration that reduces bacterial growth by ≥95%) for SBH against *Psv* was between 1.4 to 4.7 mg/L in the presence of 10 mg/L MCE. Using 25 mg/L or 50 mg/L MCE in combination with SBH, no growth was observed for all copper-sensitive and moderately-resistant strains. Inhibition against copper-resistant strains, however, was not greatly improved as compared to using 10 mg/L MCE.

1b. Efficacy of copper-SBH mixtures for the management of olive knot caused by copper-sensitive and -resistant strains of* Psv *in field studies. Field studies were initiated in the spring of 2017 on two olive cultivars in experimental or commercial plantings, and treatments are shown in Tables 1 and 2. DAS 1 and DAS 2 are SBH derivatives. DAS 2 is pre-formulated and includes copper. ZTD is a derivative of amino-thiadiazole (ATD) containing zinc that we previously tested in-vitro and that enhanced the efficacy of copper. Treatments were applied to lateral and leaf scar wounds on olive twigs, allowed to dry, and then inoculated with copper-sensitive or -resistant *Psv* strains at 10^7 CFU/ml. Results of these trials were collected in the fall, and data are currently being summarized for the annual report. On leaf scars, preliminary results

indicated that copper-ZTD mixtures reduced disease incidence caused by a copper-sensitive strain from that of the control and copper alone. On lateral wounds, however, this treatment performed similarly to copper in reducing olive knot. Again, on lateral wounds, these treatments were similar in their performance to copper.

In the second trial in a commercial orchard, kasugamycin and kasugamycin-copper mixtures resulted in the lowest disease in both lateral and leaf scar wounds with a >90% reduction in incidence. The mixtures of copper with ZTD or DAS-1 did not improve the performance of copper alone in reducing olive knot.

In additional trials using natural leaf scars, copper and kasugamycin were highly effective reducing disease incidence by >95% when using a copper sensitive strain for inoculation. The natural leaf scar as opposed to removing leaves by hand most likely had a natural abscission zone that helped to prevent bacterial ingress.

2a. Determine the efficacy of the bio-pesticide Serenade (*Bacillus subtilis *strain QST 713) in field studies on the management of olive knot. Trial procedures were similar to those in Section ***1b***. Serenade and Serenade-copper mixtures were not effective at the rates evaluated in reducing olive not as compared to the non-treated control.

2b. Determine the efficacy of the GRAS food additives nisin, epsilon-poly-L-lysine, and lactic acid in field studies on the management of olive knot. Trial procedures were similar to section ***1b***. On leaf scars, preliminary results indicated that lactic acid reduced disease incidence caused by a copper-sensitive strain from that of the control and copper alone. On lateral wounds, however, this treatment was less effective but was statistically similar to copper in reducing olive knot. Using a copper-resistant strain, lysine, nisin, and lactic acid had the lowest disease incidence on leaf scars. Again, on lateral wounds, these treatments were similar in their performance to copper.

Table 1. Treatments and rates used in field studies to accomplish select research objectives for 2017 at an experimental olive orchard in UC Davis.

Objective	Treatment	Rate/A
1b	ChampION^{++}	3.5 lb
	ChampION^{++} + SBH*	3.5 lb + 24 oz
	ChampION^{++} + DAS 1	3.5 lb + 64 oz
	DAS 2	64 oz
	DAS 2	128 oz
	ChampION^{++} + ZTD	3.5 lb + 32 oz
	ChampION^{++} + Manzate Prostick	3.5 lb + 2.4 lb
1b	ChampION^{++}	2 lb
	ChampION^{++} + SBH*	2 lb + 24 oz
	ChampION^{++} + DAS 1	2 lb + 64 oz
	ChampION^{++} + ZTD	2 lb + 32 oz
	ChampION^{++} + Manzate Prostick	2 lb + 2.4 lb
2a	Serenade Opti	20 oz
	Serenade Opti + ChampION^{++}	20 oz + 3.5 lb
2b	Nisin	1%
	Lysine	1%
	Lactic acid	1%

[1]- Treatments were applied to leaf scar and lateral wounds of Arbequina and Manzanillo olive using a hand-held sprayer until runoff, allowed to dry, and inoculated with a copper-sensitive or -resistant *Psv* strain at 10^7 CFU/ml. A total of 50 leaf scar wounds and 50 lateral wounds were made and treated for each treatment. The field study was done as a randomized complete block design and included an untreated-inoculated control.

Table 2. Treatments and rates used in a field study to accomplish objective *1b* for 2017 at a commercial olive orchard in Yuba City.

Objective	Treatment	Rate/A
1b	ChampION^{++}	3.5 lb
	ChampION^{++} + DAS 1	3.5 lb + 64 oz
	ChampION^{++} + DAS 1	3.5 lb + 128 oz
	DAS 2	64 oz
	ChampION^{++} + ZTD	3.5 lb + 32 oz
	Kasumin	1 %
	ChampION^{++} + Kasumin	3.5 lb + 1 %

[1]-Treatments were applied to leaf scar and lateral wounds of Arbequina olive using a hand-held sprayer until runoff, allowed to dry, and inoculated with a copper-sensitive *Psv* strain at 10^7 CFU/ml. A total of 50 leaf scar wounds and 50 lateral wounds were made and treated for each treatment. The field study was done as a randomized complete block design and included an untreated-inoculated control.

Supplemental efforts in 2017.

1) We published one Plant Disease article on sanitizing field equipment using quaternary ammonium.

2) We submitted a manuscript to Plant Disease on the efficacy of kasugamycin for managing copper-sensitive and -resistant strains of the pathogen causing olive knot.

3) We are currently preparing a third manuscript on the epidemiology of the olive knot pathogen.

Contains confidential information for the COC. Please do not post online.

Department of Botany and Plant Sciences
Relevant AES/CE Project No.: 4556

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL PROGRESS REPORT

Project Year: 2017

Anticipated Duration of Project: New 2-year proposal to determine the efficacy of PGR and pruning treatments to manage alternate bearing; this requires yield data for 2 consecutive years.

Project Leaders:

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Project Title: Managing Alternate Bearing in Olive with PGRs and Pruning

Cooperators:

Lindcove REC — 'Manzanillo' table olive orchard, Lindcove

Proposal Goal, Objective and Research Plan: This project is based on our discovery of the four mechanisms by which the ON-crop of olive fruit reduces return bloom the following year and perpetuates alternate bearing in 'Manzanillo' olive trees. The ON-crop causes: (1) inhibition of summer vegetative shoot growth; (2) inhibition of spring bud break; (3) abscission of floral buds; and (4) inhibition of floral development. Whereas, these mechanisms are typically discussed based on the effects of the ON crop, keep in mind the OFF crop has the opposite effect for each mechanism. Taken together, the four negative effects of the ON-crop on return bloom, especially the abscission of more than 70% of the floral buds for next year's bloom and the inhibition of floral development caused by the ON crop of fruit, made it abundantly clear that early fruit thinning (before pit hardening) would be necessary to mitigate alternate bearing in 'Manzanillo' olive. Moreover, fruit thinning would improve the efficacy of PGR treatments that increase summer vegetative shoot growth and spring bud break to increase floral intensity following the production of the ON crop. This project also utilizes what we have learned about the timing and efficacy of PGR treatments that we have tested as branch injections and whole tree sprays. Further, current year treatments were modified based on the results obtained in Year 1 of this research.

The PGRs included in the current year's experiment are: (*i*) 6-benzyladenine applied pre-bud break in February (6-BA, Maxcel®, Valent BioSciences$_{TM}$), a cytokinin, to increase spring bud break and inflorescence number of olive trees going into an OFF bloom and an OFF-crop year, i.e., these olive trees were ON-crop trees last year and, in the summer of the ON-crop year were treated with 6-BA to increase summer vegetative shoot growth and the number of nodes that can bear inflorescences at spring bloom; (*ii*) aminoethoxyvinylglycine applied at 10% open flowers (AVG, ReTain®, Valent BioSciences$_{TM}$), an ethylene biosynthesis inhibitor, to reduce flower and fruit drop to increase fruit set by the OFF bloom and increase yield of the putative OFF-crop year, these trees were ON-crop trees last year; (*iii*) 1-naphthaleneacetic acid applied at full bloom to the east side of each tree (NAA, ALCO® Olive Stop, AMVAC Chemical Corporation), a fruit thinning agent, to reduce fruit set by olive trees going into an ON bloom and to reduce yield of the putative ON crop year in order to increase fruit size during the ON-crop year and increase yield the following year (these trees were OFF-crop trees last year), which will be left unpruned to determine if the crop gets switched from the west side of the tree this year to the east side next year in order to even out alternate bearing; (*iv*) NAA applied at full bloom to the east side of the tree followed by pruning of the east side of the tree to shift the bloom from the west side of the tree this year to the east side of the tree next year (the efficacy of treatments *iii* and *iv* will be compared to determine if NAA alone is sufficient to reduce crop load and also stimulate summer vegetative shoot growth to increase flowering the following year); (*v*) OFF-crop control trees; and (*vi*) ON-crop control trees. All treatments were applied to a single tree in each block of uniform yielding trees. There were 14 blocks and 6 treatments (i.e., 14 individual trees per treatment in a randomized complete block design). In addition, we also tested a new proprietary product for fruit thinning being developed by Valent BioSciences on a separate set of ON-bloom 'Manzanillo' trees in a different block (Treatment *vii*). The thinning effect of this new material should be less sensitive to temperature and give more uniform results from year to year than NAA. The PGR treatments were applied to 'Manzanillo' olive trees in a block, which included 'Barouni' olive trees as the pollenizer planted at a ratio of one to ten, at the Lindcove REC in Exeter, CA; the trees had been lightly hand-pruned to maintain space and sunlight within rows and between rows in Year 1 (no light, no flowers).

The goal of our research is to develop a flexible management practice that can be adapted to ON- and OFF-bloom trees to even out alternate bearing in 'Manzanillo' olive orchards, so that growers do not experience the dismally low yields of an OFF-crop year. The approach will shift yield from one side of the tree to the other every other year, using chemical thinning and/ or pruning +/- PGR treatments so that yield each year will be greater annually than the average of the ON- and OFF-crop years and fruit size each year giving maximum yield of commercially valuable size fruit, i.e., solving the problem of small fruit size of the ON-crop year.

2017 Progress to Date: ON- and OFF-crop 'Manzanillo' olive trees in the orchard at the Lindcove REC were selected in Year 1 of this research based on the yield history for the past 2 years and the experiment was blocked in relation to the yield history of the trees for the past 2 years. All Year 2 PGR treatments have been applied. Trees in treatments that include pruning will be pruned just before pit hardening (endocarp sclerification) at the end of June or early July. Harvest will be in October 2017, at which time we will determine the total kg of fruit per tree and collect a subsample of 100 fruit per tree, for which we will determine the weigh of individual fruit and measure the length and diameter of each individual fruit to determine the pack out (fruit size distribution) and to estimate the total number of fruit per tree for each treatment.

vs

Canopy management, tree hedging and topping to optimize yield

Introduction and scope

Mechanical hedging and topping can be important tool in improving harvest efficiencies by affecting return bloom, helping to maintain trees in their allotted space and reducing hand pruning costs. Typically, hedging and topping result in smaller and more compact trees. Smaller trees will facilitate hand harvest by obviating the need for tall, cumbersome ladders and likely increasing the number of bins harvested per hour. Picking crews have repeatedly commented that they prefer to harvest from mechanically hedged and topped trees than from traditionally pruned trees (Louise Ferguson, personal communication). In oil olive orchards, mechanical hedging has resulted in increased harvest efficiency and reduced alternate bearing (Charlie Garcia, California Olive Ranch, personal communication). However, timing of mechanical hedging is critical for optimal yields. Hedging too late in the season may not provide enough time for new shoots to grow and flower buds to initiate. Earlier work that we conducted on 'Arbequina' oil olives indicated that shoot growth that occurred after early July did not produce flowers the following year. Whether 'Manzanillo' olives will behave the same is unknown. Hedging too early in the season can cause extensive vegetative growth at the expense of fruit growth. Thus, finding 'the sweet spot' for the timing of mechanical hedging is important to maximize and help regulate yields.

Materials, methods and results

Nickels Trial

We initiated the trial in late April 2016 (Figure 1) as a randomized block design with 3 treatments and 4 replicates. The treatments were: a) 10 foot topping, b) 13 foot topping and c) control – no topping. All trees were hedged on April 25 followed by hand pruning on May 26. We measured the time it took for 7 pruners to prune 30 trees in all treatments to estimate pruning costs. The 10 foot topping treatment removed significant amounts of wood and produced shorter statured trees (Figure 2). Trees were harvested on October 13, 2017 and samples were taken to Musco Olive to evaluate fruit size and value of the crop.

Pruning costs, crop yields, price (based on the grading sheet) and partial economic return (calculated as the product of yield and price with pruning costs subtracted) are presented in Table 1. Trees that were topped at 10 feet resulted in pruning costs that were about half the non-topped control. No significant differences ($p < 0.05$) were found between olive yields in 2016 or 2017; however, trees topped at 10 feet produced lower cumulative yields than trees topped at 13 feet and the non-topped control. Trees topped at 10 and 13 feet produced larger fruit than the control, resulting in a great price per ton (Table 1). This greater value, however, could not compensate for the lower olive yields. The partial economic returns were greatest in the control treatment.

Light levels were measured in the tree canopy throughout the season in 2017 (Figure 3 and 4). On a typical day, light levels measured at 1.5 meter from the ground (lower canopy) were

significantly greater in trees that were topped at 10 feet than trees topped at 13 feet (Figure 5). The smaller trees caused less shading in the lower canopy and likely increased fruit size compared with trees topped at 13 feet.

Hedging Timing Trials

Nielsen Trial

A major goal of these trials is to determine the most effective timing of canopy hedging to ensure return bloom and minimize excessive vegetative growth. Another important goal is to evaluate hedging effects on alternate bearing. In oil olive, hedging reduces the severe yield swings in alternate bearing trees. The experiment was established as a randomized block design with 4 repicates in a 14 year-old orchard at Erik Nielsen's farm. In 2017, monthly hedging began on March 1 and ended May 3 (Table 2).

Hedging influenced fruiting intensity, fruit set, and yield in 2017 (Table 2). Severe hedging decreased fruiting intensity but increased fruit set. Severe hedging in 2016 decreased yields in 2017. These data indicate that severe hedging not only decreased yields in 2016 but also in 2017. These carryover yield effects were not found with moderate hedging. Trees that were moderately hedged in 2016 produced similar yields to the non-hedged control in 2017.

Timing of tree hedging influenced vegetative and reproductive growth (Figure 7). Trees that were hedged earlier in the season produced greater shoot growth and number of inflorescences than trees that were hedged later in the season. Trees that were hedged after mid-July produced no inflorescences the following year. This indicates that early spring hedging is recommended for shoot growth and return bloom the following year.

Burreson Trial

In Spring 2017, we initiated a new trial on 8-year old olive trees at Heath Burrison's orchard (Figure 4). The trial was set up as a factorial design with four hedging dates and two canopy sides (east or west; the orchard is planted in a north-south direction) and replicated 5 times. The 10-tree plots were hedged on March 8, April 5, May 8, and June 8, 2017. Trees tend to grow slightly more on west-facing canopies and on canopies facing east. Thus, we want to evaluate the effect of canopy orientation on yield and canopy growth.

Shoot growth from trees hedged in March and April was significantly greater than growth from trees hedged in May and June and the non-hedged control. However, no yield differences were found among the hedging dates (data not shown). We are currently evaluating the grade sheet data.

Additional Activites

'Manzanillo' olives have been collected from various olive orchards and are currently being dried. Following drying they will be ground and set for nutrient analyses at UC Davis. These data will be used to develop a nutrient removal calculator for 'Manzanillo' olives.

We will be collecting light levels in both orchards using the UC Davis mule at the end of the season. These data and shoot growth measurements will be used to access the regrowth of the orchard following hedging treatments.

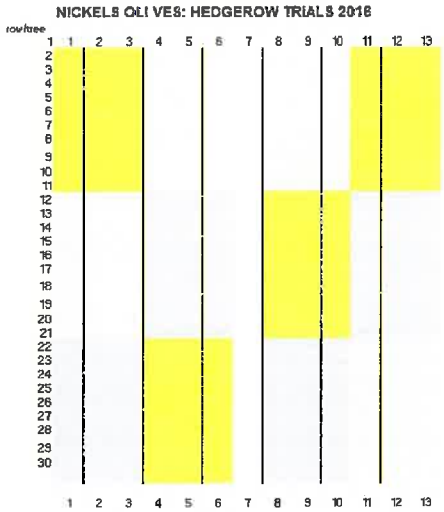

Figure 1. Set up of Nickels trial. Yellow = 10 foot topping followed by hand pruning to remove stubs with thinning cuts; Green = 13 foot topping followed by hand pruning to thin canopy and remove stubs; Blue = Hand pruned. Solid line represents where double boom hedger traveled in May 25, 2016 (5 feet from trunk).

Figure 2. Trees following 10 foot topping and hedging 5 feet from the trunk.

Figure 3. Measuring light levels using a point PAR sensor in the canopy (left) and using a quatum sensor (right) following hedging

Figure 4. Students installing point PAR sensors in the canopy in June 2017.

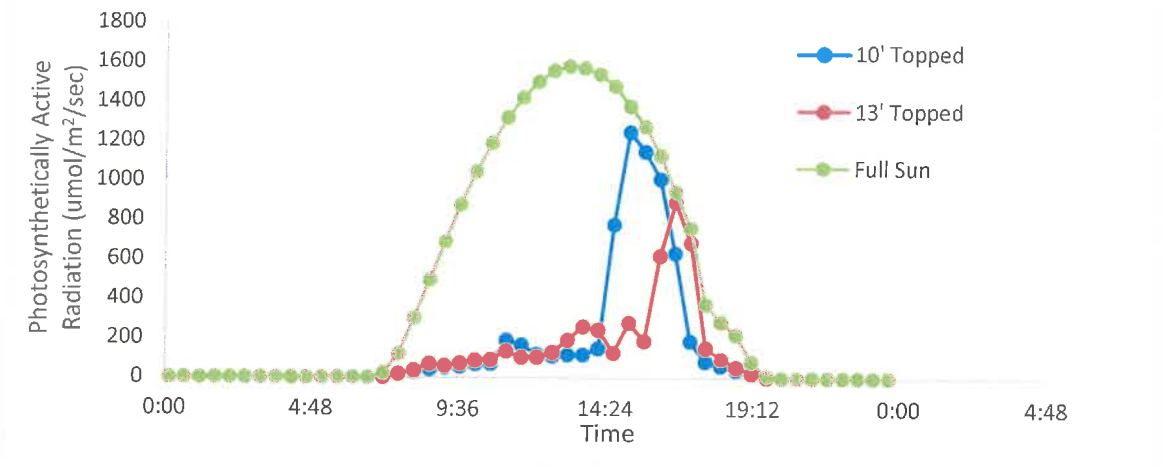

Figure 5. Light levels at full sun (green), in a 10'(blue) and a 13' topped tree taken on the west-facing canopy at 1.5 meters from the grown (lower canopy) on September 9, 2017.

Figure 6. Set up of Nielsen trial in Orland, California. Colors correspond to the following hedging dates:

Black = 27-Apr -16	Blue = 15-Jul-16 Severe	Blue Pokadot= 24-May-16 Severe
Green = 24-May-16	Pink = 27-Apr-16 Severe	Orange = 15-Jul-16
Red/White = 1-Mar-17	Purple = 29-Mar-17	Yellow = 3-May=17
White = Control		

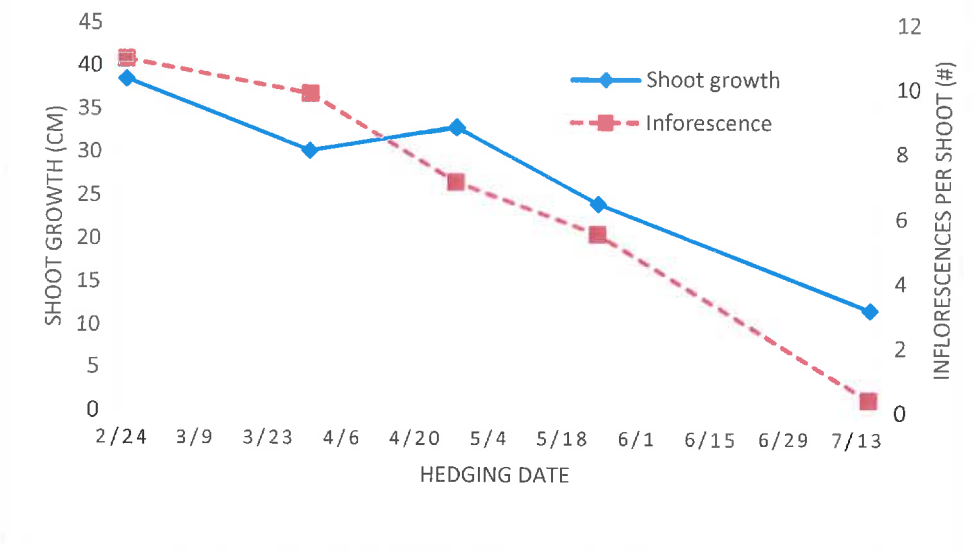

Figure 7. Relationship between hedging date and shoot growth and number of inflorescences per shoot taken on May 12, 2017.

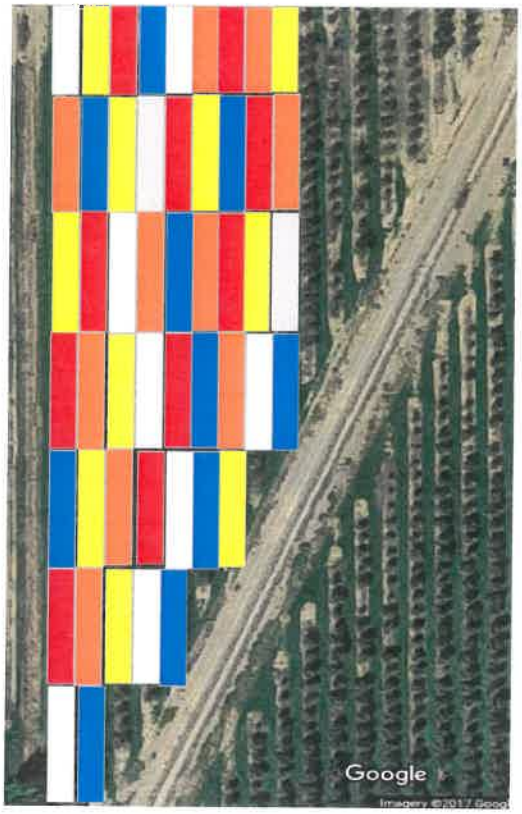

Figure 8. Hedging timing trial located at Heath Burreson's orchard, Orland, California. Trees hedged at the following times: Yellow = 8-Mar, Red = 5-Apr, Blue = 8-May, Orange = 8-Jun, and White = No Hedge Control. Experiment is set up as a factorial design with 5 hedging timings and 2 hedging positions (east and west) with 5 replicates.

Table 1. Relationship between topping height and pruning costs, 'Manzanillo' olive yields, fruit value, and return at Nickels farm.

Treatment	Pruning Costs 2016* ($/a)	Pruning Costs 2017 ($/a)	Pruning Cost Cumulative	Yields (t/a) 2016	Yields (t/a) 2017	Yields Cumulative (t/a) (2016 -2017)
Topped at 10'	500 a**	237 a	737 a	2.01	3.78	5.79 b
Topped at 13'	885 b	317 b	1202 b	3.57	5.27	8.64 a
Control	930 b	304 b	1234 b	4.65	4.37	8.63 a
P value	0.045	0.026	0.017	0.091	0.241	0.016

*pruning costs based on time needed to prune the trees multiplied by $11/hr.
**different letters in the same column indicate significance $p < 0.05$.
** partial economic return was calculated as the product of yield and price with pruning costs subtracted, no other costs were included

Table 2. Effects of hedging date and severity of hedging on 'Manzanillo' olive yields and flowering.

Hedging Date	Severity of Hedge*	% Light Inter-ception 2016	Yield (lbs/a) 2016	Flowering Intensity[3]	Fruit Set per 20 Inflores-cence	Yield Ranking 2017
No Hedge	NA	85 a	13246 a	2.6 a	6.1	7.0 ab
24-May-16	Moderate	74 b	14533 a	2.3 a	6.8	7.4 a
27-Apr-16	Moderate	76 b	14313 a	2.3 a	6.8	7.1 ab
15-Jul-16	Severe	68 cb	12070 ab	2.2 a	7.4	5.3 bc
15-Jul-16	Moderate	73 b	10528 ab	2.2 a	5.9	6.3 ab
27-Apr-16	Severe	71 b	6183 b	0.81 b	7.5	6.7 ab
1-Mar-17	Moderate				6.2	5.1 bc
29-Mar-17	Moderate					4.3 c
3-May-17	Moderate					5.1 bc
P value		0.037	0.041	0.0043	0.09	0.0001

* Moderate = approximately 8.5 feet from trunk; Severe = approximately 6.5 feet from trunk
3 Rating 5 = very heavy flowering, all branch have flowers present over the full length of the canopy; 0 = no flowers present

Preliminary field study to identify new olive fly control materials

Project Leader: Dr. Dani Lightle, Orchards Advisor, Glenn, Butte & Tehama Counties
Cooperating Personnel: Dr. Robert Van Steenwyk, Entomology Specialist Emeritus, UC Berkeley
Dr. Emily Symmes, Area IPM Advisor, Sacramento Valley

Overview:
This research study proposes a preliminary evaluation of three full cover materials (Assail, Sivanto, and Venerate) for olive fly control. Separately, it evaluates three materials (Danitol, Venerate and Grandevo) in a reduced volume application. We plan to submit the most promising full cover material to IR-4 for further evaluation in fall 2017. Marrone Biosciences (maker of Venerate and Grandevo) will move forward with adding olive to their labels if efficacy is demonstrated.

Objectives & Methods:
1. Evaluate the field efficacy of three full-cover materials: Assail 30SG, Sivanto 200SL and Venerate XC

A field site was established in the Kopta Slough area near Woodson Bridge (Sacramento River) in Tehama county. This area is known for high olive fly pressure relative to the surrounding region. The orchard is Sevillano olive at a 20' x 20' spacing. Each treatment was 9 trees (3x3 tree square plots); each treatment was replicated four times. McPhail traps baited with Torula bait were set on June 5th, 2017, prior to pit hardening. The trap was placed on the center tree in each plot and checked roughly every 7 days. Bait was replaced at each trap check.

The treatments were applied by Dr. Bob Van Steenwyk using a hand gun. All treatments were applied in 150 gal water/ac and 9 gal molasses/ac. The treatments were:

- Molasses alone (control)
- Venerate XC, 4 qts/ac
- Sivanto 200 SL, 14 oz/ac
- Assail 30SG, 8oz/ac
- Danitol 2.4EC (grower standard)

Treatments were applied at pit hardening (June 28-29, 2017) and later in the season once trap counts began to increase (August 29-24, 2017).

Trap counts for this site are shown in Figure 1. Trapping is ongoing and olives will be picked prior to harvest and evaluated for olive fly stings.

2. Evaluate the field efficacy of two low-volume treatment materials: Venerate XC, Grandevo WDG, and Danitol 2.4 EC.

A field site was established in the Woodson Bridge area near the Sacramento River in Tehama county. Parts of this orchard were not picked last year because the olive fly damage was too great. The orchard is Sevillano olive at a 20' x 20' spacing. Each treatment was 9 trees (3x3 tree square plots); each treatment was replicated four times. McPhail traps baited with Torula biat were set on June 5th, 2017, prior to pit hardening. The trap was placed on the center tree in each plot and checked roughly every 7 days. Bait was replaced at each trap check.

The treatments were applied with a Lil' Squirt air assist sprayer (PBM Supply & Mfg., Inc, Chico,CA) pulled by a quad at a rate of 10gal/ac and 9 gal molasses/ac. The treatments were:

- Molasses alone (control)
- Grandevo WDG, 3 lbs/ac
- Venerate XC, 4 qts/ac
- Danitol 2.4EC, 7.1 fl oz/ac

- GF-120 (grower standard)

Treatments were applied beginning June 12, 2017 and applied approximately every 2.5 weeks (16-19 days).

Trap counts for this site are shown in Figure 2. Trapping is ongoing and olives will be picked prior to harvest and evaluated for olive fly stings.

Preliminary Conclusions:
So far, fly catches have been much lower this summer than the previous few years. This may be because of the greater number of days over 100˚F than typical for the region. Fly catch data will be analyzed for differences between treatments at each site after the fruit are harvested. Additionally, olives will be checked for olive fly stings and differences in damage will be compared between treatments.

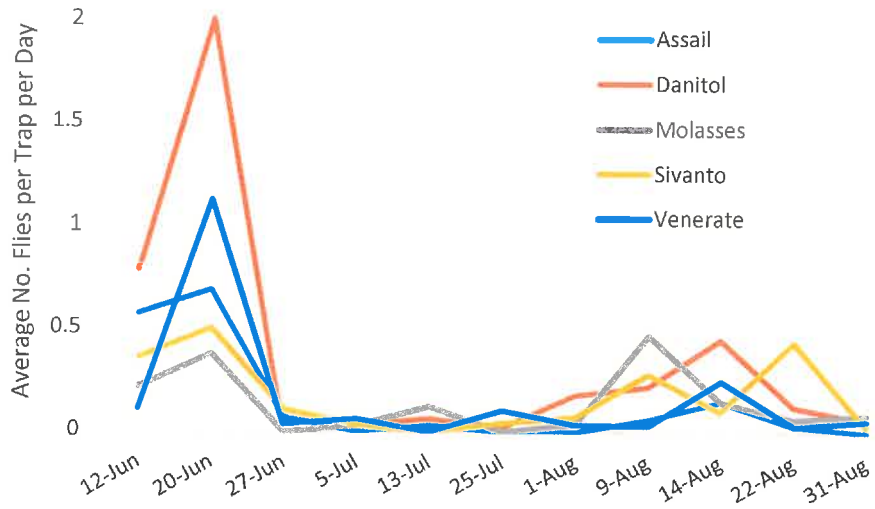

Figure 1. Average number of flies trapped per day to date in each treatment for the full cover spray site in Tehama county. Overall, fly counts are much lower this year than previous summers, possibly because of the many consecutive days over 100˚F.

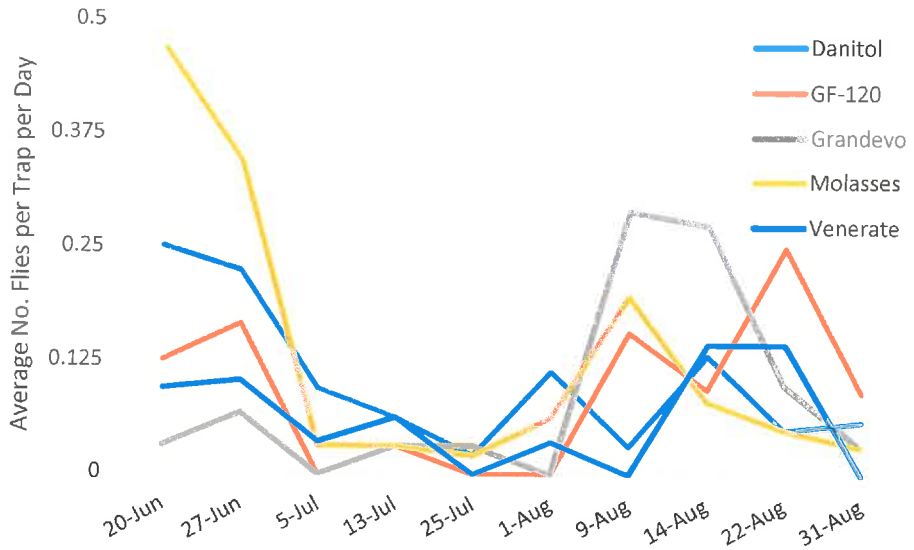

Figure 2. Average number of flies trapped per day to date in each treatment for the reduced volume spray site in Tehama county. Overall, fly counts are much lower this year than previous summers, possibly because of the many consecutive days over 100°F.

Summary of 2018 Proposals to the California Olive Committee

2018 Proposals to COC		
Title	**PI**	**COC Budget**
A new fruit removal head for an olive harvesting system	R. Ehsani	$45,741
Canopy management, tree hedging and topping to optimize yield	R. Rosecrance et al.	$31,075
Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard	Preece and Ferguson	$35,442.75
Managing alternate bearing in olive with PGRs and pruning	C. Lovatt & E. Fichtner	$20,698
Evaluation of several promising addatives for reducing acrylamide in black ripe table olives.	S. Wang	$53,280
Differentiation of olive cultivars based on DNA and NMR-based fingerprinting methods.	S. Wang	$67,433
Novel insecticide controls for black scale: general impact and non-target concerns.	H. Wilson & K. Daane	$20,454
New materials for olive fruit fly	D. Lightle	$25,000*
Southern San Joaquin Valley Olive Fruit Fly Monitoring Project	J. Stewart	$6,400
Sacramento Valley Olive Fruit Fly Monitoring Project	E. Simpson	$6,500
Epidemiology and management of olive knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi*	J. Adaskaveg	$16,650
* budget estimate; actual budget pending results of current year's project.	Total	$303,674

A new fruit removal head for an olive harvesting system. Funding is requested for first year of a two year project. Goal of project is to develop canopy shaker with an articulated frame to improve canopy adaptability and reduce shaking intensity. This would be a smart shaker head equipped with sensors. PI: R. Ehsani

Canopy management, tree hedging and topping to optimize yield. The goals of these experiments are to determine the most effective timing of canopy hedging and topping height to ensure return bloom, maximize yields, and minimize excessive vegetative growth. PIs: R. Rosecrance, W. Krueger, L. Ferguson, D. Lightle

Canopy management, tree hedging and topping to optimize yield. PIs request funding for maintenance and data collection from established research block designed to investigate dwarfing potential of rootstocks.PIs: J. Preece, L. Ferguson.

Managing alternate bearing in olive with PGRs and pruning. Investigating use of pruning techniques and foliar application of plant growth regulators for mitigation of alternate bearing and maintenance of economically valuable fruit size. PIs: E. Fichtner &C. Lovatt.

Evaluation of several promising additives for reducing acrylamide in black ripe table olives. Identify compounds that can be added during the processing of table olives to reduce the acrylamide in the final product. PI: S. Wang

Differentiation of olive cultivars based on DNA and NMR-based fingerprinting methods. Project will develop a genomic database for black ripe olives and allow for DNA-based identification of olives. The result will allow for identification of imported olives at a cultivar level. PI: S. Wang

Novel insecticide controls for black scale: general impact and non-target concerns. Funding is requested for the first year of a two year project. Project focuses on investigating efficacy of insecticides for management of black scale and determining influence of these products on non-target organisms. PIs: H. Wilson and K. Daane

New materials for olive fruit fly. Current submission is a placeholder proposal; PI is still analyzing data from 2017 study and will subsequently plan for the 2018 season. Investigating efficacy of full coverage applications of Asail, Sivanto, and Venerate for management of OFF. Also investigating low volume applications of Venerate, Grandevo and Danitol. PI: D. Lightle.

Southern San Joaquin Valley Olive Fruit Fly Monitoring Project. Provide information on adult OFF populations to assist with timing of management of this important pest. PI: J. Stewart

Sacramento Valley Olive Fruit Fly Monitoring. PI. E. Simpson

Epidemiology and management of olive knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi*. Funding is requested for the 2nd year of a three year proposal. Budget is split 50:50 between the COOC and the COC . Primary goals of project are to develop new management techniques for management of olive knot disease and further registration of effective compounds. PI: J. Adaskaveg.

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: School of Engineering – Mechanical Engineering

Project Year: 2018 Anticipated Period of Performance: 03/01/2018 – 02/28/2019

Project Title: A New Fruit Removal Head for an Olive Harvesting System

Project Leaders: Reza Ehsani (Professor, University of California, Merced, 5200 N. Lake Road, Merced, CA 95343, 209-228-3613, rehsani@ucmerced.edu)

Cooperators: Louise Ferguson (CE Pomologist, Department of Plant Sciences, UC Davis, Email: lferguson@ucdavis.edu, Phone: (559) 737 3061)

Commodity: Olive
Relevant AES/CE Project No.:

Year Initiated: 2018
Anticipated Duration of Project: 2 years (only one year proposed here)

Problems and Significance:
Production acreage of table olives, California's signature crop, has significantly decreased in recent years due to the high cost of production and small margin of profit. Harvesting is a major cost of production for table olives. Currently, the majority of table olives are hand-harvested. Although some growers are using trunk shakers with some success, this method has not been widely utilized because older trees that have larger trunks cannot be harvested by trunk shakers. Also, growers are hesitant to remove and replace high yield producing older trees with younger trees. Mechanical harvesting, using contact canopy shakers, is the most promising method for harvesting table olives. Scientists at UC Davis have developed a prototype of a canopy shaker that has been tested and has shown some level of success. The UC Davis-designed canopy shaker is very similar to the canopy shaker used in harvesting process oranges in Florida. This type of shaker is relatively heavy and cannot accommodate the shape of the tree very easily, plus research at the University of Florida has shown that fixed shaking frequency is not the best choice for maximum fruit removal and a better fruit removal can be achieved if a range of frequencies be used. In spite of all past efforts, there is still a need for a cost-effective and efficient harvesting system to match the needs of existing table olive trees.

Progress to Date:
Mechanical harvesting of olives was initiated in the US in 1940s. The main goal of this method was to develop a cost-effective technique to harvest olive fruit for both table and oil extraction purposes (Sola-Guirado *et al.*, 2014). Among all proposed methods, mechanical shaking has been the most successful approach for fruit removal. Different types of shakers such as a trunk shaker, branch shaker and canopy contact shaker were developed (Jimenez-Jimenez *et al.*, 2015 and

Famiani *et al.*, 2014). To increase the efficiency of using these shakers, previous research studies suggested high density hedgerow orchards with limited tree height. Trunk shakers had lower fruit removal efficiency due to the damping effect of branches (Castro-Garcia *et al.*, 2014 and Fergusen *et al.*, 2014). Beside the lower efficiency, damage to the bark of the trunk and branches cause lower yield in the future, and increase the risk of infestation and disease in the trees (Jimenez-Jimenez *et al.*, 2015). For other types of shakers, especially contact canopy shakers, damage to the branches and leaves and also final fruit quality issues such as cuts and flesh injury should be taken in to consideration (Fergusen *et al.*, 2010). All these damages reduce the market acceptability, especially of green processed table olives. To solve the issues with mechanical harvesting of traditional orchards, Fergusen *et al.*, 2010 suggested to consider modifications in both canopy size of traditional trees and mechanical harvesters simultaneously. In this project, we are proposing a new canopy shaker configuration with an articulated frame to improve the canopy adaptability and reduce the shaking intensity and damage to both tree and fruits for conventional orchards. This will be a smart shaker head and will be equipped with sensors to self-adjust its shaking frequency and tine position to reduced tree damage.

Objectives:

The ultimate goal of this project is to reduce the harvesting cost for table olives. The specific goal is to develop a cost-effective fruit removal system for existing conventional olive trees with higher harvest efficiency and better fruit removal percentage while reducing damage to the fruit and tree branches. This is a two-stage project, the first stage includes developing a new fruit removal mechanism and installing it on a test platform and conducting field trials to evaluate its performance. If successful, then we will propose the second stage in which we will compare the new designed fruit removal head with the current shaking mechanism developed by UC Davis researchers for conventional olive trees.

Experimental Procedures:

To achieve the objectives of the proposed project, the following tasks should be done. Tasks for each objective are listed below individually.

Task 1- Review the literature to find the issues with introduced harvesters and visiting the orchards with conventional trees to determine the specific requirements of a proper fruit removal head.
Task 2- Design and build the head in the shop and install the head on a test platform.
Task 3- Conduct both stationary and dynamic tests to make sure all mechanical components work properly and field evaluation to verify the head functionality. The field evaluations consist of percentage of fruit removal, evaluation of damage to the tree branches and leaves and visual evaluation of damage to the fruit.
Task 4- Write a final report for the first year of the project and summarize the results of machine evaluations.

Anticipated Outcomes:

It is anticipated that the newly designed harvesting head is cost-effective and lighter than the previous design and will cause less damage to the fruit making it more suitable to be used on the existing table olive trees.

Select References:

Castro-Garcia, S., Castillo-Ruiz, F. J., Jimenez-Jimenez, F., Gil-Ribes, J. A., & Blanco-Roldan, G. L. (2015). Suitability of Spanish 'Manzanilla'table olive orchards for trunk shaker harvesting. Biosystems Engineering, 129, 388-395.

Famiani, F., Farinelli, D., Rollo, S., Camposeo, S., Di Vaio, C., & Inglese, P. (2014). Evaluation of different mechanical fruit harvesting systems and oil quality in very large size olive trees. Spanish Journal of Agricultural Research, 12(4), 960-972.

Ferguson, L., Rosa, U. A., Castro-Garcia, S., Lee, S. M., Guinard, J. X., Burns, J. & Glozer, K. (2010). Mechanical harvesting of California table and oil olives. Advances in Horticultural Science, 53-63.

Ferguson, L., Burns, J., Miles, J., Guinard, J. X., Rosa, U., Castro-Garcia, S. & Vossen P. M. Developing Mechanical Harvesting for California Black Ripe Processed Table Olives: 2006-2014.

Jimenez-Jimenez, F., Blanco-Roldana, G.L., CastilloRuiza, F. J., Castro-Garcia, S., Sola-Guiradoa, R., Gil-Ribesa, J.A. (2015). Table Olives Mechanical Harvesting with Trunk Shakers. Chemical Engineering, 44.

Sola-Guirado, R. R., Castro-García, S., Blanco-Roldán, G. L., Jiménez-Jiménez, F., Castillo-Ruiz, F. J., & Gil-Ribes, J. A. (2014). Traditional olive tree response to oil olive harvesting technologies. Biosystems Engineering, 118, 186-193.

BUDGET REQUEST: Reza Ehsani
Budget Year: 2018

Funding Source: COC

Labor:	**(Line 1)**	**$26,916 +$1,292**
Salary – 2 Graduate Student Researcher, 100% for 3 months of summer 2018, $4,489 per month		$26,916
Benefits – 4.8%		$1,292
Subtotal 1	**Line 1 subtotal**	**$28,208**
Supplies, Equipment:	**(Line 2)**	**$10,000**
Supplies: (hauling the mechanical harvester to the field ($1,500), renting a platform for installing the harvesting head for two weeks ($2000) raw materials for fabrication ($1,500), hydraulic components, hosing and hydraulic motors, hydraulic valves ($3,000), consumable shop and welding supplies, supplies for field data collection supplies ($2,000)		$10,000
Equipment: N/A		$0
Individual contractors: N/A		$0
Subtotal 2	**Line 2 subtotal**	**$10,000**
Travel:	**(Line 3)**	**$380+$2,620**
Vehicle Use: (Renting a truck for two weeks for field trials $380 ($190.00/week)		$380
Meeting attendance: (*attending COC meeting ($320)* attending a professional meeting for one person Registration + airplane ticket and accommodation $2,300)		$2,620
Subtotal 3	**Line 3 subtotal**	**$3,000**
Subcontracts: N/A	***(Line 4)***	**$0+$0**
Collaborator A: N/A		**$0**
Collaborator B: N/A		**$0**
Subtotal 4	**Line 4 subtotal**	**$0**
Total of lines 1 through 3 above	**(Line 5)**	**$41,208**
UCD/ANR/UCR Overhead @ 11% IDC	**Line 6**	**0.11 * Line $41,208**
Total to primary PI	**(Line 7)**	**Line 4 $0 + Line 6 $4,533**

TOTAL BUDGET REQUEST **$45,741**

PRIMARY PI SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

Reza J Ehsani
Originator's Signature

10/31/2017
Date

Department Chair/County Director

10/31/17
Date

Caroline Lutz
Liaison Officer

11/01/2017
Date

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Olive / Plant Sciences College of Agriculture, CSU Chico

Project Year 2018 Anticipated Duration of Project: 4 years

Project Title: Canopy management, tree hedging and topping to optimize yield

Project Leaders:

Rich Rosecrance, Professor, California State University, Chico. College of Agriculture, 400 West First Street, Chico, CA 95929-0310: rrosecrance@csuchico.edu

William H. Krueger: Glenn County Farm Advisor (Emeritus): whkrueger@ucanr.edu

Louise Ferguson, Extension Specialist, Department of Plant Sciences, 2037 Wickson Hall, Mail Stop II, UC Davis, 1 Shields Ave., Davis CA 95616, (530) 752-0507 [Office], (559) 737-3061 [Cell] LFerguson@ucdavis.edu

Daniele Lightle: Glenn County Farm Advisor: DLightle@ucanr.edu

Cooperating Ranches and People:

Erik Nielsen Enterprises Inc. 4453 Co Rd O, Orland, CA 95963

Dennis Burreson, Musco Olives, 17950 Via Nicolo, Tracy, California 95377

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2016 Current Funding Request: **31,075.00**

Problems and Significance:

Mechanical Hedging

Mechanical hedging and topping can be important tool in improving harvest efficiencies by affecting return bloom, helping to maintain trees in their allotted space and reducing hand pruning costs. Typically, hedging and topping result in smaller and more compact trees. Smaller trees will facilitate hand harvest by obviating the need for tall, cumbersome ladders and likely increasing the number of bins harvested per hour. Picking crews have repeatedly commented that they prefer to harvest from mechanically hedged and topped trees than from traditionally pruned trees (Louise Ferguson, personal communication). In oil olive orchards, mechanical hedging has resulted in increased harvest efficiency and reduced alternate bearing (Charlie

Garcia, California Olive Ranch, personal communication). However, timing of mechanical hedging is critical for optimal yields. Hedging too late in the season may not provide enough time for new shoots to grow and flower buds to initiate. Earlier work that we conducted on 'Arbequina' oil olives indicated that shoot growth that occurred after early July did not produce flowers the following year. Whether 'Manzanillo' olives will behave the same is unknown. Hedging too early in the season can cause extensive vegetative growth at the expense of fruit growth. Thus, finding 'the sweet spot' for the timing of mechanical hedging is important to maximize and help regulate yields.

Mechanical Topping

Unlike hedging, mechanical topping does not reliably produce a crop on shoots that grow in response to the topping. Our trials have demonstrated that topping produced vigorous growth with limited fruit and resulted in two problems: 1) The limited fruit in the upper canopy ripened sooner than the rest of the crop, producing overripe fruit that decreased grade and value by 25%, and 2) Vigorous vegetative growth that can shade fruitwood and decrease yields, even when the tree is topped every other year. The solution appears to be to top the tree annually with a gabled cut to eliminate this overly vigorous growth and overripe fruit.

Optimizing Tree Light Interception at different tree heights, and latitudes

A program that evaluates light interception at different tree heights, row spacings, and latitudes has been developed by David Connor in Spain. This program was developed to help determine optimal tree spacing and height to maximize light interception at different latitudes. This program will assist us in our hedging and topping treatment to increase light interception and yield.

Fruit Nutrient Removal Calculator

Significant quantities of nitrogen, phosphorus and potassium are removed by harvested portions of fruit crops. Thus, the nutrient removal rate is an important consideration for making fertilizer recommendations. Inadequate fertilization and/or nutrient imbalance can prevent growers from achieving desired fruit yields and quality. Recently, we developed a macro- and micro-nutrient removal calculator for 'Arbequina' oil olives (Figure 1). Oil olives, however, are smaller and have a greater pit to flesh ratio than 'Manzanilla' table olives, which influence fruit nutrient content. An online fruit nutrient removal calculator needs to be developed for table olives.

Progress to Date:

Nickels Soil Lab

We harvested the trial at Nickels Soil Lab in Arbuckle with a mechanical harvester on October 13, 2017. Cumulative yields (2016 and 2017) indicated that yields were reduced by almost 3 tons per acre in the 10-foot topping treatment compared with trees topped at 13 feet and the no

topping treatments, 5.8, 8.6, and 8.6 tons per acre, respectively. The 10-foot topping treatment, however, reduced hand pruning cost by almost $500 per acre compared with the 13-foot and no topping treatments, likely the result of a more compact tree. Currently we are analyzing light levels and fruit size along a transect from the base to the top of the tree. In the unhedged treatment, preliminary data indicate that fruits located between 1 and 1.5 m from the ground were significantly smaller that fruit higher up in the canopy. No difference in fruit size along the transect was found in the 10-foot topping treatment. This likely resulted in the greater percentages of large fruit in the 10-foot vs the 13-foot and hand pruned treatments. We will be evaluating how topping affect pruning costs, return bloom, and yields in 2018

Nielsen Trial

Trees hedged in 2016 still had reduced canopies in late 2017 compared with a non-hedged control. Severe hedging in 2016 increased shoot growth and decreased reproduction in 2017 compared with a non-hedged control. Severe hedging (removal ~ 30% of the canopy) in 2016 significantly reduced flower intensity and fruit set and increased shoot growth in 2017. In contrast, flower intensity and fruit set levels were similar among the moderately hedged and non-hedged treatments. As in 2016, yield-ranking data in 2017 indicated that severe hedging reduced yields, likely a result of carbohydrates being diverted to shoot growth rather than to fruits. It will be interesting to see how 2016 hedging treatments affects yields in 2018.

Burrison Trial

In Spring 2017, we initiated a new trial on 8-year old olive trees at Heath Burrison's orchard (Figure 2). The trial was set up as a factorial design with four hedging dates and two canopy sides (east or west; the orchard is planted in a north-south direction) and replicated 5 times. The 10-tree plots were hedged on March 8, April 5, May 8, and June 8, 2017. Trees tend to grow slightly more on west-facing canopies and on canopies facing east. Thus, we want to evaluate the effect of canopy orientation on yield and canopy growth. Shoot growth from trees hedged in March and April was significantly greater than growth from trees hedged in May and June and the non-hedged control. Yields will be collected in the near future.

Olive Nutrient Removal Calculator

At fruit maturity, fruit samples were collected from eight orchards up and down the state. Fruits dried, grown, and analyzed for maco- and mico-nutrients at Dellavalle Labs, Fresno, California. We will use these data and data collected in 2018 to develop a nutrient removal calculator.

Objectives:

We propose to:

1. Investigate the effects of timing of mechanical hedging on return bloom, yield on mature trees. The objective is to determine the optimal timing of hedging for hedgerow plantings for generating a 5-ton or more per acre annual average crop.

2. Compare the effects of a mechanical pruning program that incorporates annual topping at two different tree heights to controlling the tree height. All of the treatments would receive an every other row middle hedging. The objective is to determine the optimal hedgerow height for generating a 5-ton per acre annual average crop that can be produced with mechanical pruning. This data could then be used to evaluate the program for determining optimum tree height for hedgerow plantings.
3. Compare results from hedging and topping trials with the a MatLab program which predicts optimal tree size and spacing to maximize light interception.
4. Develop a web-based fruit nutrient removal calculator for 'Manzanillo' table olives

Experimental Procedures:

Experiment 1: Mechanical Hedging (Erik Nielsen's and Heath Burreson's orchard)

Hypothesis: optimal timing or mechanical hedging will not decrease yield and will facilitate mechanical harvesting.

Overall Objective: to determine the optimal timing of mechanical hedging for table olive productivity and fruit quality.

2017 Objectives:

I. Hedge Trees Monthly
II. Evaluate effect of pruning treatments on shoot growth, and return bloom and quality: perfect versus imperfect flowers.
III. Evaluate effect of pruning treatments on yield and fruit quality.
IV. Determine optimal timing of hedging treatment to facilitate high quality fruit production and return bloom.

Materials and methods:

Experimental Design:
Randomized complete block of four replications.

- Treatments: Evaluate timing by hedging the south side of the tree at monthly intervals starting in April and ending in August. Twelve trees from 4 tree rows will be hedged each month.
    - Hedging will aim to remove about 50 percent of the new growth
    - middle 10 trees of each treatment will be the data trees

- Data Collection:
- 100 fruiting and 100 non-fruiting branches will be tagged after hedging treatment
- Shoot growth will be measured at the end of the seasons
- At bloom the following season, flowering intensity (inflorescences per branch) will be determined from the tagged branches
- Following bloom, fruit set will be determined

- Measure fruit removal and yields following mechanical trunk shaking in the hedged trees.

- Data Analysis:
    - The following relationships will be evaluated statistically for the trial:
        - Effect of time of hedging on shoot growth in both fruiting and non-fruiting shoots.
        - Effect of time of hedging on flowering the next year from fruiting and non-fruiting shoots
        - Effect of time of hedging on fruit set the next year from fruiting and non-fruiting shoots
        - Evaluate the effects of the treatments of fruit removal and yields following mechanical trunk shaking.

Experiment 2: Mechanical Topping

Materials and Methods:
Experimental Plot: Nickels Estate - 2 acre 'Manzanillo' orchard established in 2002.

Hypothesis: mechanically topping hedgerow olive orchards will not decrease yield and will reduce hand harvesting costs by producing shorter statured trees.

Overall Objective: to determine the optimal row height for table olive productivity and fruit quality at a 12 X 18' orchard spacing (202 trees/acre) and develop the formulas for applying this information to different latitudes and orchard spacing.

2017 Objectives:

V. Apply two different tree height pruning treatments and compare to controlling tree height with hand pruning
VI. Install sunlight exposure monitoring cameras
VII. Evaluate effect of pruning treatments on bloom quality: perfect versus imperfect.
VIII. Evaluate effect of pruning treatments on yield and fruit quality in upper and lower canopy at harvest.
IX. Correlate hours of sunlight exposure with fruit yield and quality.

Materials and methods:

Experimental Design:
Randomized complete block of four replications: map attached

- Treatments: three pruning treatments of three, 10 tree rows
    - topped at 10 and 13 feet in February 2017 and compared to pruning to lateral branches at 13 feet using thinning cuts
    - middle row of each treatment will be the data row
    - alternate row side hedging treatments will be applied

- Data Collection:

- Five photosynthetically active radiation (PAR) monitors will be positioned on a 20' pole and installed along a transect from the trunk to the top of the tree. Measurements will be taken every 5 minutes and compared with the full sun measurement. Fruit size at each position will be determined.
- A late-season mid-day light interception measurement will be done to determine the percentage of light each treatment is intercepting.
- Trees will be harvested and fruit quality will be assessed from samples taken from the upper and lower tree canopy.
- Yields will be compared with the MatLab program that predicts optimal tree size and spacing to maximize light interception.

- Data Analysis:
    - The following relationships will be evaluated statistically for the east and west sides, within the three pruning treatments:
        - Effect of pruning treatment on ratio of perfect to imperfect flowers
        - Effect of pruning treatment on total yield and fruit quality; size and color
        - Correlation of each of the above parameters with total hours of light exposure through the season from bud swelling through harvest.

Olive Nutrient Removal Calculator

At fruit maturity, fruit samples will be collected from eight orchards up and down the state. Fruits will be dried, grown, and analyzed for maco- and mico-nutrients. We will use these data to develop a nutrient removal calculator. Growers will input their olive yield and this web-based tool will determine the amount of macro- and micro-nutrients removed in the harvested crop, similar to what is shown in Figure 1.

Anticipated Outcomes:

Hedging and Topping Treatments

The goal of these experiments are to determine the most effective timing of canopy hedging and topping height to ensure return bloom, maximize yields, and minimize excessive vegetative growth. We anticipate that hedging and topping treatments can producer similar yields to hand-pruned trees with lower labor costs. We also anticipate the hedging and topping will significantly reduce alternate bearing.

Light Measurements in topped, hedged, and control trees

The goal of these experiments are to determine how canopy management with mechanical topping and hedging affects total hours of canopy light exposure and therefore flower production, fruit yield and quality. The ultimate goal is to demonstrate how to calculate the optimal tree height for moderate density orchards at different latitudes.

Olive Nutrient Removal Calculator

The 'Manzanillo' nutrient removal calculator will estimate nutrient removal of macro- and micro-nutrients. Removal data for the 'Manzanillo' will be incorporated into the calculator found at http://www.csuchico.edu/~rrosecrance/Model/OliveCalculator/OliveCalculator.html

BUDGET REQUEST –

Budget Year: 2018-2019	Funding Source: COC
Personnel:	
Rich Rosecrance, California State University, Chico, professor.	6,000.00
data collection and entry, harvest support. (~87 hrs @ $69/hr)	
Student (summer and fall; 375 hours at $12/hr)	4,500.00
Fringe @ 11.01%	1,156.05
Independent Contractor - Bill Krueger: Glenn County Farm Advisor (emeritus):	6,000.00
Technical Support - data collection and entry, harvest support.	
Sub 1	**17,656.05**
Equipment Supplies & Expenses:	
Light measurement, timelapse cameras, field scale equipment	3,600.00
Sub 2	**3,600.00**
Pruning and Harvesting Costs: (based on previous year's cost)	
Hand pruning, brush shredding: Nickels Estate	1,500.00
Mechanical harvest (ENE Inc.) at Nickels Estate:	1,500.00
Hand harvest at Nickels Estate (post mechanical harvest)	1,000.00
Nutrient Analyses (18 samples x $56/sample)	1,000.00
Miscellaneous harvest supplies: water, gloves, tarps, buckets	1,000.00
Sub 3	**6,000.00**
Experimental Travel Costs:	
Travel support for plot set-up, data collection, harvesting. (8 months X 4 RT/month @ 120 miles/trip X .55/mile)	2,338.95
Sub 4	**2,338.95**
Facilities and Admin @ 5%	**1,480.00**
TOTAL BUDGET	**31,075.00**

Scope of Work

Dr. Richard Rosecrance:
Responsible for overall coordination of the project, applying pruning treatments, executing harvest trials, developing fruit nutrient calculator, data collection and analysis and writing final report.

Bill Krueger, Louise Ferguson, and Dani Lightle: Responsible for assisting in the mechanical pruning treatment in Orland and Nickels trial and co-coordinator of harvesting the trials.

External Contractors: contracts to be secured after funding.

Pruning Contract at Nickels Soils Laboratory: Colusa, California
Hillary Nielsen Porter
ENE Inc.
4453 County Road O
Orland CA 95963
ENE@EneInc.com
Office: 800-844-9409
FAX: 530-865-4845

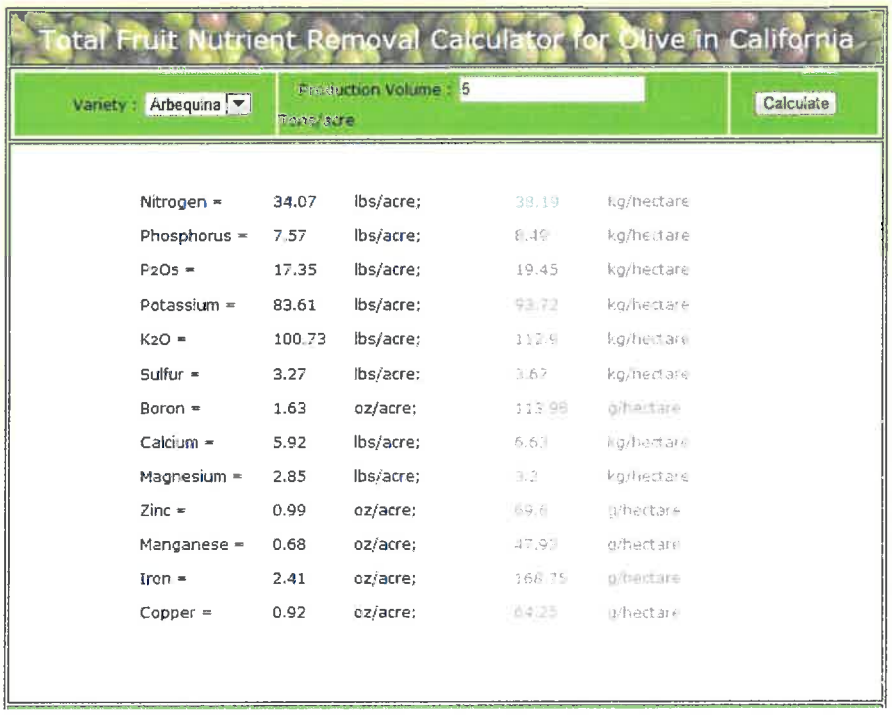

Figure 1. Nutrient removal calculator for 'Arequina', 'Arbosana', and 'Koroneiki' olive oil cultivars. Data will be collected to include 'Manzanillo' in the fruit nutrient removal calculator.

Figure 2. Hedging timing trial located at Heath Burreson's orchard, Orland, California. Trees hedged at the following times: Yellow = 8-Mar, Red = 5-Apr, Blue = 8-May, Orange = 8-Jun, and White = No Hedge Control. Experiment is set up as a factorial design with 5 hedging timings and 2 hedging positions (east and west) with 5 replicates.

PRIMARY PI SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

Originator's Signature	Date
Department Chair/County Director	Date
Liaison Officer	Date

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Olive / Plant Sciences, UC Davis

Project Year 2018 Anticipated Duration of Project: 10 years (5 of 10)

Project Title:
Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard
April 1st – December 31st 2018 (5th year timeframe)

Project Leaders:
Dr. John Preece: Research Leader, USDA-ARS National Clonal Germplasm Repository, UC Davis, 1 Shields Ave., Davis CA 95616. John.Preece@ars.usda.gov, (530)-752-7009
Dr. Louise Ferguson: Extension Specialist, Department of Plant Sciences, 2037 Wickson Hall, Mail Stop II, UC Davis, 1 Shields Ave., Davis CA 95616, (530) 752-0507 [Office], (559) 737-3061 [Cell], LFerguson@ucdavis.edu
Mr. Dan Flynn; University of California Olive Center, Davis CA
JDFlynn@UCDavs.edu; (530)-752-5170
Mr. James M. Jackson: Principal Superintendent, Plant Sciences Field Facility, UC Davis CA
JMJackson@ucdavis.edu; (530)-753-2173 and (530)-681-2279

Commodity: Olive Relevant AES/CE Project No.

Year Initiated: 2013 Current Funding Request: **$35,442.75**

Problems and Significance:
To facilitate mechanical harvesting the newest table olive orchards are planted in hedgerows and require regular mechanical pruning to keep the trees small. Our 12 X 18' foot research planting established at Nickels Soils Laboratory in 2002 has demonstrated to us this will be difficult with the 'Manzanillo' olive cultivar. Such hedgerow 'Manzanillo' orchards designed for mechanical harvesting would be easier to maintain if they could be grafted on dwarfing rootstocks. Among those olives with promise for use as a dwarfing rootstocks are:
Nikitskaya,
Olea cuspidate
Verticillium Resistant Oblonga
Dwarf D
Little Ollie (2015 addition)

In 2013 we proposed propagating these rootstocks and testing with grafted and non-grafted own rooted 'Manzanillo' controls for their dwarfing potential with 'Manzanillo' to produce a tree that is more amenable to mechanical harvesting. The own rooted 'Manzanillos' and 'Manzanillo' grafted to 'Manzanillo' in this orchard could also serve as the next generation hedgerow trained mechanically pruned orchard for mechanical harvesting with trunk and canopy contact shakers.

In 2013 year we were awarded funding to propagate the desired rootstocks and locate a suitable orchard site for establishment of the propagated trees. Both objectives have been achieved but due to difficulty of propagation with some cultivars and difficulty in locating a site with proper infrastructure planting was in spring 2014.

Progress through 9/30/2017:

This application for initial funding was for two purposes:

I. **Propagation and grafting of the rootstocks with 'Manzanillo' scions.**
   a. Dr. John Preece supervised the development of specific propagation techniques for 112 each of the following olive cultivars to be used as dwarfing rootstocks; Nikitskaya, *Olea cuspidate,* Verticillium Resistant Oblonga and Dwarf D. Dwarf D proved very difficult to root as cuttings and this means that there were sufficient trees only for the closer spacing. At the wider spacing, Little Ollie, which roots easily is being tested, which adds another potential rootstock and expands the scope of the study in a logical way.

II. **Establishing the next generation olive hedgerow orchard for evaluation of mechanical harvesters.**
   a. Field 3556, a four acre block located in Plant Sciences Field Facility located on the UC Davis Campus and maintained by UC Davis Plant Sciences field personnel was chosen as the planting site. This site has the added advantage of being located adjacent to oil orchards being developed by the UC Olive Center. The trees were planted in 2014. The plants were grafted in 2015 and pruned to nurse limbs and grafts in 2016 and to the graft scions in 2017.

III. **Experimental Field Design:**
   a. Split plot design with the north half of the field at spaced at 10 X 16' and the south at 10 X 8'.
   b. There are 4 Randomized Complete Blocks
   c. Four different dwarfing rootstocks grafted with 'Manzanillo'
   d. Own rooted 'Manzanillo' and 'Manzanillo' grafted to a 'Manzanillo' grafting controls.
   e. Sevillano pollinizers were planted as border rows around the perimeter of the orchard and in the middle, as a row between the wide and narrow spacing.

Progress Summary:

The trees planted in 2014 were maintained and staked and grown through the summer of 2015 to allow the trees to reach sufficient size for grafting. The 'Oblonga' trees were falling over more and in more need of staking (which was done) than the others. In spring of 2015, the border rows of 'Sevillano' pollinizers were completed by planting the last 41 trees. There were insufficient trees available in 2014 to complete the border rows.

Some of the rows of dwarf olives were incomplete, therefore additional cuttings were rooted and trees produced at the National Clonal Germplasm Repository nursery. The exception is that 'Dwarf D' has proven to be extremely difficult to root to produce plants for the wider spacing portion of the study. Therefore, in addition, cuttings of 'Little Ollie' were rooted and this

cultivar proved to be easy to propagate. On September 29 2015 the nursery produced plants were planted into the orchard and 'Little Ollie' replaced the originally planned 'Dwarf D' at the wider spacing. This completes the planting and also gives a fifth genetically different rootstock to test for dwarfing of olive. One of the 'Sevillano' trees died during the summer of 2015, but there were a few extra trees from the spring 2015 planting, and that tree was replaced. Sierra Gold Nursery and staff of the National Clonal Germplasm Repository grafted the trees from September 28 – Oct. 1, 2015. This will give a cooler time of the year for the grafts to heal and take. Following grafting, the orchard was sprayed with Kocide to control olive knot.

The grafted trees and controls were pruned back to the grafted shoots, plus nurse limbs on May 10, 2016. Since then, the trees have been maintained by regular sprays with Kocide for olive knot, skirting for ease of herbicide application, and fertilization. On July 10, 2017, the trees were pruned to their scions.

2018 Objectives:

I. **Finish grafting any rootstocks without grafts, in the spring.**

II. **Collect data to study the any growth differences among the scions on the different rootstocks compared to the controls**

Experimental Procedures:
Complete grafting. This will be completed in spring, 2018.

The goal is to be able to dwarf the olive trees by using one or more of these rootstocks. Therefore, data will focus on measurements of vegetative vigor, including branch numbers and lengths, tree height, tree caliper of both the rootstock and scion. Yield data, if available, will be collected.

Data will be analyzed using ANOVA with an LSD means separation.

Desired Result:
At maturity the rootstocks will maintain tree size at 10 feet or less, and the trees can be harvested with trunk shakers or canopy contact harvesters. The experimental design will also allow a determination of 'Manzanillo' tree yields at 10 X 16' and 8 X 16' feet spacings.

BUDGET REQUEST: 2018
BUDGET REQUEST
Budget Year: 2018

Funding Source: COC

Orchard Maintenance Costs:	**4,600.00**

UC Davis Plant Sciences Field Facility: 4 acres @ 650.00/acre per year:	2,600.00
Chemicals; herbicide, Kocide, NPK fertilizer, vertebrate and insect pest control:	1,000.00
Supplies: switch drip to micro-irrigation system, replacement stakes:	1,000.00
Sub 1	**4,600.00**
Data Collection:	**14,175.00**
.25% of a Junior Specialist @ 3129.00/month + .51% benefits = 1,182/month X 12 months =	14,175.00
.25% of truck rental/gas for 12 months estimated @ 600.00/month =	1,800.00
1 month (133 hours) of student assistance for data collection @ 15.00/hour =	2,000.00
Sub 2	**22,575.00**
UC Davis Overhead @ 11%	**12,867.75**
TOTAL BUDGET REQUEST	**35,442.75**

UNIVERSITY OF CALIFORNIA

	Originator's Signature	10/15/2016 Date
Agricultural Experiment Station	Department Chair	Date
	Liaison Officer	Date

Scope of Work

Drs. John Preece, Louise Ferguson and Mr. Dan Flynn:
Responsible for overall coordination of the project and orchard management.

Mr. James M. Jackson:
Responsible for orchard management implementation.

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Olive Workgoup/Department of Botany & Plant Sciences, UCRiverside

Project Year: 2018 — Anticipated Period of Performance: 2 crop years to assess treatment effects on cumulative yield of alternate bearing olive trees

Project Title: Managing Alternate Bearing in Olive with PGRs and Pruning

Project Leaders: Elizabeth Fichtner and Carol Lovatt

EF-Farm Advisor, Orchard Systems, Cooperative Extension, 4437 S. Laspina St., Tulare, CA 93274; Phone: 559-684-3310; Fax: 559-685-3319; Email: ejfichtner@ucanr.edu

CL-Professor of Plant Physiology, Emeritus & Professor in the Graduate Division, Botany and Plant Sciences-072, UCRiverside, CA 92521-0124; Phone: 951-827-4663; Fax: 951-827-4437; Email: carol.lovatt@ucr.edu

Cooperators: Kurt Schmidt, Lindcove Research and Education Center, 22963 Carson Avenue, Exeter, CA 93221; Phone: 559-592-2408, ext. 153; Email: krschmidt@ucanr.edu

Commodity: Olive — Relevant AES/CE Project No.: 4556-H

Year Initiated: 2018 — Anticipated Duration of Project: 2 crop years

Problems and Significance: Alternate bearing (AB), production of a heavy "on-crop" (high yield, ON-trees) followed by a light "off-crop" (low yield, OFF-trees), occurs in perennial fruit and nut crops, as well as forest species (where it is called "masting"). For tree crops, alternating high and low yields cause significant economic problems. In ON-years, trees produce a large number of small size fruit with reduced commercial value. In OFF-years, trees produce large fruit, but in some cases a significant proportion of the fruit are too large and have reduced economic value, further exacerbating the problem that there are too few fruit in OFF-crop years to provide growers with a good income. For olive, the ON-crop takes longer to mature, attain size and accumulate oil. The delayed harvest further reduces floral intensity the following spring. It is important to note that the lack of fruit in the OFF-crop year has a negative economic impact on every step in the production chain from farm to consumer, including orchard management, harvesting, packinghouse operation, manufacture of value-added products, marketing, and consumer prices, which jeopardizes the stability and sustainability of tree-crop commodity-based industries.

The severity of alternate bearing in the olive industry in Tulare County is illustrated in Table 1. Since the major factor initiating AB is an extreme climate event (high or low temperature, etc.) that ultimately reduces yield and initiates AB, there is a reoccurring need for a management strategy to mitigate the severity of AB.

Progress to Date: a) *Understanding the mechanisms that perpetuate AB in olive.* Our research identified four mechanisms by which the ON-crop of olive fruit reduces return bloom, and thus

yield, the following year and perpetuates AB in 'Manzanillo' olive trees (Fichtner et al., 2017). The ON-crop causes the following, with the OFF-crop having the inverse effect. (1) The setting ON-crop of developing olive fruit inhibits summer vegetative shoot growth as first reported by Sibbett (2000) and confirmed by our research. This reduces the number of nodes that can produce floral buds at spring bloom the following year. In addition, our COC-funded research also documented that spring vegetative shoot growth is reduced when trees produce an ON-bloom, reducing the number of inflorescences contributed by the spring vegetative shoots to bloom the year following the ON-crop (Fichtner and Lovatt, 2016). (2) Despite being harvested 4 to 6 months before spring bud break, the ON-crop of olive fruit inhibits spring bud break, which also reduces inflorescence number. (3) From fruit set to harvest, the ON-crop of fruit causes abscission of floral buds, with the greatest abscission occurring in September, a mechanism we identified in our COC-funded research that was previously only known to function in AB pistachio trees (Chao, 2014; Fichtner et al., 2017). (4) The ON-crop of 'Manzanillo' olive fruit inhibits floral development by inhibiting transcription of key genes necessary for flower formation (Chao, 2014; Fichtner et al., 2017). Our COC-funded research results also documented that the ON-crop of fruit reduces the number of inflorescences contributed to return bloom by bearing shoots (shoots that set fruit) to a greater degree than non-bearing shoots (shoots that did not set fruit). Bearing shoots are negatively impacted by both the fruit set on the shoot (localized effect of fruit) and all the fruit on the tree (whole tree crop load effect), whereas non-bearing shoots on ON-crop trees are influenced only by crop load. As a result, non-bearing shoots are the major source of inflorescences at spring bloom following the ON-crop year. Thus, increasing the number of non-bearing shoots on ON-crop trees by fruit thinning (before pit hardening) is essential for reducing the negative impact of the four mechanisms that reduce flowering and perpetuate AB. Moreover, fruit thinning should improve the efficacy of PGR treatments that increase vegetative shoot growth and spring bud break on non-bearing shoots to increase floral intensity and yield in the year following the production of the ON-crop. This project also utilizes what we have learned about the timing and efficacy of PGR treatments that we have tested as branch injections and whole and half tree sprays.

b) *Progress in the use of PGRs to mitigate AB in olive.* For 'Manzanillo' olive, injection of 6-benzyladenine (6-BA) or adenosine (ADO) (alone or combined with tri-iodobenzoic acid [TIBA]) into scaffold branches of ON-crop 'Manzanillo' olive trees in July significantly increased summer vegetative shoot growth for non-bearing shoots of ON-crop trees to a value equal to that of non-bearing shoots of OFF-crop control trees and significantly greater than that of bearing shoots of ON-crop control trees ($P < 0.0001$) (Table 2) (Fichtner et al., 2017). However, only ADO (alone or combined with TIBA) significantly increased summer vegetative shoot growth on bearing shoots of ON-crop olive trees to a value equal to that of non-bearing shoots of OFF-crop control trees ($P < 0.0001$). For 'Manzanillo' olive, combining ADO with TIBA provided no benefit over using ADO alone to increase summer vegetative shoot growth. Injecting these same compounds into the scaffold branches of a second set of ON-crop 'Manzanillo' olive trees in February demonstrated that both 6-BA and ADO had a positive effect on spring bud break and floral intensity at return bloom (Fichtner et al., 2017). As a result, 6-BA and ADO increased the number of inflorescences produced by non-bearing shoots of ON-crop trees to values significantly greater than those of non-bearing shoots on both OFF- and ON-crop control trees at return bloom ($P < 0.0001$) (Table 2). Supplying TIBA with ADO reduced the benefit of ADO alone. All three treatments increased inflorescence number at return bloom for bearing shoots of ON-crop 'Manzanillo' olive trees relative to bearing shoots of ON-crop control trees, but not to the level of non-bearing shoots of either ON- or OFF-crop trees ($P < 0.0001$) (Table 2), confirming the need to increase the number of non-bearing shoots on ON-crop trees.

c) *Progress with PGR strategies and increasing the number of non-bearing shoots on ON-crop 'Manzanillo' olive trees obtained from our most recent COC-funded research.* ON-crop trees receiving a summer application of 6-BA last year (July 2016) to increase summer vegetative shoot growth were treated with 6-BA again in February 2017 to increase spring bud break and floral intensity at bloom. We continued to test AVG applied at 30% bloom to trees going into an OFF-bloom. Neither treatment increased yield to values greater than control trees going into an OFF-bloom; all produced OFF-crops that were significantly lower than control trees going into an ON-bloom and producing an ON-crop ($P < 0.0001$). In year 2 (2017) of our current COC-funded project, based on the results presented above supporting the need to increase the number of non-bearing shoots on ON-crop trees and the large number of ON-crop trees present in our research orchard, as proposed we tested the efficacy of the PGR naphthaleneacetic acid (ALCO® Olive Stop™; AMVAC Corp.) applied at full bloom at the manufacture's suggested rate to just one side of ON-crop 'Manzanillo' olive trees to reduce bloom and fruit set in the ON-crop year in order to increase spring and summer vegetative shoot growth in the current year and spring bud break, floral intensity and yield on that side of the tree the following year so that it would not produce an OFF-crop. By chemically thinning only half of the tree, the impact of over-thinning on yield when a heat wave occurs is reduced. During the last week of June, we pruned (mechanically hedged) one side of a second set of ON-crop trees to compare the efficacy of chemical fruit thinning versus mechanical pruning on yield and fruit size of the current year's harvest and of next year's return yield following the ON-crop. For fruit removal by pruning, pruning was delayed to the end of June to enable growers to evaluate the crop set by their trees or the efficacy of NAA, if it was applied at bloom, in order to make an informed decision to prune or not, and how severely to prune. Removing fruit by pruning at this time is sufficiently early to promote summer vegetative shoot growth and to have a positive effect on floral bud retention and floral gene expression and spring bud break. Moreover, mechanical hedging is typically a less expensive method of pruning. All trees were topped during the first week of July 2017. In this strategy, the side of the tree that was not treated with NAA or not pruned is the source of the current year's crop, with an increase in average fruit size anticipated relative to fruit of untreated ON-crop control trees. However, the untreated side will produce an OFF-bloom and an OFF-crop the following year, although some increase in return bloom and yield is anticipated on this side of the tree due to the reduction in total fruit number per tree (crop load) on the treated side of the tree. The proposed strategy directs the grower to treat the other side of the tree the following year with NAA or pruning, based in the intensity of the bloom or crop set by June. Thus, each year, alternating sides of the tree would be treated with NAA or pruned. In our research, NAA and mechanical pruning (hedging) reduced yield equally, 28% and 23%, respectively, resulting in yields that were significantly lower than the yield of ON-crop control trees, but significantly greater than the yield of OFF-crop control trees by more than 2-fold ($P < 0.0001$). In addition, the results confirmed that removing fruit on one side of the tree significantly increased average fruit size for the whole tree. NAA and pruning increased fruit size by 20% and 15% compared to ON-crop control trees, respectively, but the fruit were still 10% and 15% smaller than fruit of OFF-crop control trees, respectively ($P < 0.0001$). The average size of fruit on OFF-crop trees was extra large (82 fruit/lb), for ON-crop trees, medium (111 fruit/lb) and for the NAA and pruned trees, large (93 fruit/lb). In a second orchard, we tested two concentrations of the new proprietary material (1-aminocyclopropane-1-carboxylic acid, ACC, a precursor of ethylene biosynthesis) from Valent BioSciences, the action of which might be less sensitive to high temperature, possibly reducing the potential for over-thinning in a heat wave. The treatment was also applied to only one half of the tree. The two concentrations of the proprietary fruit-thinning PGR ACC reduced yield 36% and 31% compared to ON-crop

control trees, but the effects were not statistically significant do the low number of replications possible in this small set of trees. The ACC treatments did not increase average fruit size per tree numerically or statistically. For all fruit thinning treatments, return yield data for next year is critical for determining the capacity of the treatments to even out yield in an AB orchard and establish the degree of thinning that is required. Imposing the treatments on alternate sides of the trees annually and collecting yield data over multiple years is essential to determine if good yields can be maintained and AB mitigated.

Two additional items of information. First, Dr. Fichtner observed during the collection of fruit samples for fruit size determination that the number of black and partially colored fruit was possibly treatment related. We evaluated this possibility using the fruit samples we collected for each data tree. She was correct. There was a strong inverse relationship between the proportion of black and partially black fruit per tree and total yield per tree (r = - 0.60; $P < 0.0001$), with OFF-crop control trees having the largest proportion of black fruit (31%) per tree ($P < 0.0003$) with another 15% partially colored fruit and the fewest green fruit (54%) ($P < 0.0003$). Conversely, the proportion of fruit that remained green through harvest increased in parallel with total yield per tree ($r = 0.60$; $P < 0.0001$) with the majority (90%) of the fruit remaining green on ON-crop control trees and a statistically equal proportion remaining green on NAA (81%) and pruned trees (75%) ($P < 0.0003$). A greater proportion of green fruit might be another benefit from evening out AB. Second, just prior to harvest we tested our ability to evaluate the effectiveness of the fruit-thinning treatments. The treated sides of the trees were rated on a scale from 0 (no crop present, 100% crop reduction), 1 (25% of crop present; 75% crop reduction) to 4 (100% of crop present; 0% crop reduction). The correlation between the crop load rating for each tree and the harvested yield per tree was positive and strong ($r = 0.71$; $P = <0.0001$). The next step is to test the rating system in June for use as a tool to facilitate the grower's decisions related to pruning.

Objectives: The overall goals of the proposed research are three-fold: (1) to even out AB so there is a good crop annually by switching crop production from one side of the tree to the other side of the tree annually; (2) to sustain even production so growers have a stable and good income annually; and (3) to provide growers with means mitigate AB when it reoccurs. These goals will be achieved by meeting the following objectives. Objective 1: To reduce crop load (total number of fruit per tree) by removing flowers and/or fruit starting in an ON-crop year by applying a PGR thinning agent at bloom and/or by pruning (hedging) before pit hardening (June) to one side of the tree only for either treatment to create more non-bearing shoots during the ON-crop year that flower and produce crop the next year. Thereafter, flowers or fruit are removed on alternating sides of the tree annually by either using a chemical thinning agent at bloom or by pruning (hedging) at bloom or in June (before pit hardening). This strategy is designed to achieve yields that are significantly greater than OFF-crop yields but less than ON-crop yields annually (at the present time, we estimate a 30% reduction of the ON-crop yield is necessary to eliminate the OFF-crop year). Objective 2: To compare the efficacy of NAA versus ACC to reduce bloom reliably from year to year to even out alternate bearing. Objective 3: To compare the efficacy of using PGRs to thin flowers versus pruning (hedging) to remove fruit in June (before pit hardening). Objective 4. To determine the effectiveness and added value (cost-benefit) of applying 6-BA to trees that have had the ON-crop removed on one side of the tree to increase summer vegetative shoot growth during the ON-crop year and stimulate bud break the spring following the ON-crop

Experimental Procedures: The experiment includes six treatments in a randomized complete block design with 15 individual trees (replications) per treatment. The treatments are: (1) ON-

crop (untreated) control trees; (2) OFF-crop (untreated) control trees; (3) ON-crop trees treated with NAA at full bloom in 2017 on one side of the tree and now treated on the other side of the tree with NAA at full bloom in 2018 and the alternate side in 2019; (4) ON-crop trees pruned in June 2017 (before pit hardening) and now pruned on the other side of the tree in June 2018, with the alternate side pruned in 2019; (5) ON-crop trees treated with ACC at full bloom in 2018 and the alternate side in 2019; and (6) On-crop trees pruned (hedged) in June 2018 and treated with 6-BA in July 2018 to stimulate summer vegetative shoot growth and 6-BA in February 2019 to stimulate spring bud break in order to test the efficacy of the PGR treatment on trees that now have an increased number of non-bearing shoots; the alternate side will be pruned in 2019 and the 6-BA treatments applied as described. The amount of summer vegetative shoot growth will be compared for treatment 6 (pruned plus 6-BA) versus treatment 4 (pruned only). Treatment effects on floral intensity, crop load and summer vegetative shoot growth will be determined for all data trees. Treatment effects on final yield and average fruit size, fruit size distribution (pack out) and the proportion of black versus green fruit will be determined at harvest. This experiment needs to run for a minimum of two years in order to evaluate cumulative yield over at least one complete AB ON/OFF cycle.

Anticipated Outcomes:

- We will gain insight into the capacity of NAA and ACC applied at full bloom to thin flowers uniformly each spring and maintain a uniform yield for successive years.
- We will have data on the impact of different degrees of fruit thinning on only half of the tree on total yield, which will enable us to estimate the impact that over-thinning due to a heat wave would have on final yield, i.e., we will have some indication of the risk associated with the application of chemical thinning agents to only half of the tree.
- We will learn whether the application of NAA or ACC supports better summer vegetative shoot growth and return bloom and return yield than June pruning (hedging).
- We will learn whether delaying pruning (hedging) to June to give growers the opportunity to evaluate their potential crop load to make a decision to prune or not to prune or how severely to prune is efficacious or has negative consequences.
- Specifically, we will learn whether pruning in June is effective or too late for stimulating summer vegetative shoot growth to increase return bloom and yield.
- Through these comparisons, potential benefits can be verified, e.g., fruit removal in June before pit hardening increases floral bud retention and flowering, or potential problems can be identified, e.g., June pruning causes loss of carbohydrates resulting in poor shoot growth, small fruit size or too much shoot growth, leading to competition and small fruit size (potential problems not encountered thus far with June pruning).
- Documentation of whether or not foliar application of 6-BA to pruned trees during the summer stimulates vegetative shoot growth and increases return bloom and return yield better than pruning alone, i.e., a cost-benefit analysis of using 6-BA with pruning.
- Data to support or refute (1) that reducing crop load on one side of the tree starting in the ON-crop year using PGRs thinning agents or pruning (hedging) increases yield in the following OFF-crop year sufficiently to even out AB and provide growers with a good annual income the following year and (2) that reducing crop load on alternate sides of the tree each year will sustain good yields and grower income annually.
- The data will document whether removing fruit on one-side of the tree increases return bloom and yield the other side of the tree, the untreated side, the following year as well, due to the reduction in crop load on the treated side of the tree.

- The harvest data will quantify the effect that removing fruit on one side of the tree has on average fruit size, fruit size distribution (pack out), proportion of black versus green fruit, and crop value.
- We anticipate a positive outcome indicating that strategy proposed above can successfully mitigate alternate bearing each time it is initiated by an adverse climate event, or other event, that results in an OFF-crop followed by an ON-crop.

Select References:

Agricultural Commission/Sealer. 2016. Tulare County Annual Crop and Livestock Reports for 2009, 2010, 2011, 2012, 2013 and 2014. (agcomm.co.tulare.ca.us/default/index.cfm/standards-and-quarantine/crop-reports1/).

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Fichtner, EJ and CJ Lovatt. 2016. Alternate bearing in olive. Acta Hort. (in press)

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Sibbett, S. (2000). Alternate bearing in olive trees. California Olive Oil News. *3* (12),1

Table 1. Olive production in Tulare County, California, 2008-2014.[z]

Year	Yield (kg/ha)	Value (US$/ha)	ABI[y] (0-1.0)
2008	4,057	685	
2009	897	186	0.6
2010	16,208	2400	0.9
2011	4,080	758	0.6
2012	7,958	1494	0.3
2013	10,491	1894	0.1
2014	341	664	0.9

[z]Adapted from Agricultural Commission/Sealer (2016).
[y]ABI = alternate bearing index = the absolute value of year 1 yield minus year 2 yield divided by the sum of year 1 yield + year 2 yield. An ABI of 0, means no alternate bearing; an ABI of 1, means complete alternate bearing, crop one year and no crop the other year.

Table 2. Effects of 6-benzyladenine (BA), adenosine (Ado) and ADO plus tri-iodobenzoic acid (TIBA) injected into scaffolding branches of ON-crop trees in July on summer vegetative shoot growth or in February on the number of inflorescences at spring bloom for non-bearing and bearing shoots of ON-crop 'Manzanillo' olive trees.

		Year 1	Year 2
Tree status	Shoot status	Summer shoot growth (no. of node pairs/shoot)	Inflorescences (no./shoot)
OFF	non-bearing	3.3 a[z]	15.4 b
ON	non-bearing	0.7 cd	13.3 b
+ 6-BA	non-bearing	2.6 ab	22.0 a
+ Ado	non-bearing	3.5 a	22.2 a
+ Ado + TIBA	non-bearing	3.6 a	15.8 b
ON	bearing	0.6 d	0.8 d
+ 6-BA	bearing	1.9 bc	4.1 c
+ Ado	bearing	2.6 ab	5.1 c
+ Ado + TIBA	bearing	2.4 ab	4.9 c
P-value		< 0.0001	< 0.0001

[z]Means in a vertical column followed by different letters are significantly different at specified *P*-values by Fisher's LSD Test.

BUDGET REQUEST: (Carol J. Lovatt)

Budget Year: 2018 Funding Source: COC

Labor:	**(Line 1)**		**$4309**
Salary: T Khuong @ $4,879/mo x 30% = $1,464; Lab Asst. 1 @ $16.47/hr x 70 hr = $1,152		$A	$2,616
Benefits: TK= $1,464 x 1.1351% = $ 1,662; Lab Asst. 1 = $1,152 x 2.65% = $31		$B	$1,693
Subtotal 1	**Line 1 subtotal:**		**$4,309**
Supplies, Equipment:	**(Line 2)**		**$6446**
Supplies: *(be specific. Examples include tape, tags, buckets, traps, safety, chemicals, etc)*		$C	$0
Equipment: (*be specific. Examples include balances, meters, devices, etc*)		$D	$0
Individual contractors: Recharge to Lindcove REC – use of olive orchard, irrigation, weeding, pruning, pest control, application of PGRs = $4,068 (based on 50% @ the old recharge rate of $16.26/h and 50% @ the new recharge rate effective July 1, 2018, of $30/h. Harvest @ $2,500 (estimated to be less than last year due reduced crop load per tree due to fruit thinning and pruning on a majority of the trees)		$E	$6,446
Subtotal 2	**Line 2 subtotal**		**$10,755**
Travel:	**(Line 3)**		**$2,487**
Vehicle Use: 5 roundtrips to Exeter (520 mi x 5 = 2,600 mi x $0.6014/mi = $1,564; UCR vehicle Rental 10 days x $47.268/day = $473; $90/day per diem (Lindcove REC trailer, plus meals) for 1 person x 5 trips (1.5 days each) = $450		$F	$2,487
Meeting attendance: (*be specific. anticipated travel to meetings such as COC meetings, professional society meetings*)		$G	$0
Subtotal 3	**Line 3 subtotal**		**$13,242**
Subcontracts: Elizabeth Fichtner	**(Line 4)**		**$6,000**
Collaborator A: Elizabeth Fichtner		$H	$6,000
Subtotal 4	**Line 4 subtotal**		**$19,242**
UC R Total	**(Line 5)**		**$13,242**
UCR Overhead on $13,242 @ 11% IDC	**(Line 6)**		**$1456.62**
(Total to primary PI – Carol Lovatt)	**(Line 7)**		**$14,698.62**
TOTAL BUDGET REQUEST	**Line 4+Line 7**		**$20,698.62**

PRIMARY PI SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

Carey Lovett — Originator's Signature — 10/27/2017 — Date

Patricia Spence — Department Chair/County Director — 10/27/2017 — Date

Liaison Officer — Date

SUBCONTRACT BUDGET REQUEST: (*Elizabeth Fichtner*)
Budget Year: 2018 Funding Source: COC

Labor:	**(Line 1)**	**$4855.61**
Salary *(Junior Specialist, Narges Mahvelati, at 7% FTE)*		$3495.76
Benefits *(38.9%)*		$1359.85
Sub 1		**$4855.61**
Supplies, Equipment:	**(Line 2)**	**$200.00**
Supplies: *(be specific. general field supplies (flagging tape, pruners, buckets, gloves. etc)*		$200
Sub 2		**$5055.61**
Travel:	**(Line 3)**	**$349.80**
Vehicle Use: *(Mileage from Tulare, CA to/from Modesto for COC meetings; 280 miles round trip @ $0.535/mile. Request partial funds ($200) toward attendance of Pomology Conference in Davis in March 2018.This is approximately 1/3 of the cost of attending the meeting; other costs would be contributed by walnut and pistachio accounts to share costs across main commodities that I serve.*		*$349.80*
Sub 3	**(Line 4)**	**$5405.41**
UCD/ANR/UCR Overhead @ 11%	**(Line 5)**	**$594.59**
Sub 4 (Total Subcontract)	**(Line 6)**	**$6000.00**

(Add Line 6 to primary PI budget in subcontract section 'H' and 'I')

SUBCONTRACT SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

Originator's Signature	Date
Department Chair/County Director	Date
Liaison Officer	Date

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Food Science and Technology

Project Year: 2018-2019 Anticipated Duration of Project: March 2018 – July 2019

Project Title: Evaluation of Several Promising Additives for Reducing Acrylamide in Black Ripe Table Olives

Project Leader:
Dr. Selina Wang
University of California, Davis
Olive Center
Food Science & Tech
Phone: 530-219-1267
E-mail: scwang@ucdavis.edu

Commodity: olives

Year Initiated: 2017 Anticipated Duration of Project: March 2018 – July 2019

Problem and its Significance:

Acrylamide has been identified as a probable carcinogen by the National Toxicology Program and the International Agency for Research on Cancer. High levels of acrylamide have been found in black ripe table olives by researchers (Table 1). In a survey conducted by the US Food and Drug Administration (FDA), high amounts of acrylamide were found in black ripe table olives in US market (375-1925 µg/kg). Casado and Montaño screened 11 black ripe table olives obtained in Spain and found the levels of the acrylamide ranged from 176 to 1,578 µg/kg of olive pulp. Similarly, in our lab, we also found 288-1,192 µg/kg acrylamide in seven Spanish black ripe table olives samples.

Research Group	Acrylamide level (µg/kg)
US Food and Drug Administration	375-1,925
Casado and Montaño	176-1,578
UC Davis Olive Center Laboratory	288– 1,192

Table 1. Acrylamide levels found in recent research in black ripe table olives.

Seeking a quick and cost-effective solution to reducing acrylamide in black-ripe processing, the UC Davis Olive Center and California table olive processors in 2014 evaluated sodium bisulfate ($NaHSO_3$) -- Casado had found that addition of 25 mM $NaHSO_3$ reduced acrylamide by 37% without any impact on sensory qualities. Unfortunately, the Casado method proved ineffective when deployed at an industrial scale – in fact, we found that the amount of $NaHSO_3$ (0, 10, and 25 mM) actually increased the amount of acrylamide in black ripe table olives at 15 and 30 min sterilization times at 240°F. Moreover, since sulfites are allergens, the addition of $NaHSO_3$ may limit commercial applications for canned olives. We looked into other options.

In 2015, UC Davis Olive Center and California table olive processors evaluated how different sterilization conditions can affect the acrylamide levels in canned black ripe table olives. We concluded that lowering the sterilization temperature and shortening the sterilization time could reduce the formation of acrylamide, while continuing to achieve appropriate process bacterial lethality. However, this may not be desirable as it has the potential of slows down production significantly.

In 2017, we designed a model system to test out various additives (amino acids: L-cysteine and glycine; flavone/phenols: luteolin, naringenin, apple skin polyphenolics, grape seed and grape skin extracts) and their ability to reduce acrylamide levels at different ranges of pH (5-9) and temperature (110-130°C). We found that two amino acids showed promising results and are likely to work in an industrial scale.

We propose the use of natural additives to reduce or completely remove acrylamide in black ripe table olives. We will work with the processors to obtain controlled (no additives) and experimental samples (with various additives at different concentrations). The pH and acrylamide concentrations of these samples will be analyzed at UC Davis.

Objectives:

The objective of this proposal is to determine compounds such as amino acids and phenols can be added during processing to reduce substantially the acrylamide levels in canned black ripe table olives.

Experimental Procedures:

We will work with the processors and provide them with the additives at different concentrations so that they can be added to the brine before sterilization or other steps during processing. A control from the standard procedure without any additives will be used to establish a comparison. It has been suggested in literature that acrylamide in ripe table olives can decrease after 6 months of storage time in the presence of acrylamide-reducing additives. Therefore, we will be measuring acrylamide concentration 1, 3, 6 months and 12 months after canning.

The pH and acrylamide concentration in olives and in brine will be determined at UC Davis. Black ripe table olives (20 g) will be shaken off the brine and crushed in the mortar with a

pestle. Then 2 g of sample will be placed in a centrifuge tube and spiked with 0.5 μg d_3-acrylamide as internal standard. Water (4 mL) will then be added to the centrifuge tube. After 10 min of shaking, hexane (1 mL) will then be added, followed by another shaking for 10 min. The samples will be then centrifuged at 8,000 rpm for 10 min to separate the aqueous and hexane layers. The separated aqueous layer (lower layer) will be vacuum filtered using a 125 mL Buchner funnel. Then nitrogen will be blown on top of the filtrate to completely evaporate the hexane.

A Sep-Pak C_{18} cartridge will be activated by methanol (2 mL) followed by water (2 mL). All of the filtrate (about 4 mL) will then be loaded on the cartridge and passed through the cartridge without vacuum (about 1.5 mL/min). The filtrate will be collected and evaporated to less than 1 mL and water to which water will be added to make up to exactly 1 mL. Acrylamide determination will be performed by LC-MS/MS.

The quantification of acrylamide and d_3-acrylamide will be performed on a Sciex API 2000 triple-quadruple MS system. The samples will be separated using a Hypersil-Keystone Hypercarb column (50×2.1 mm i.d., particle size 5μ). The mobile phase will be isocratic methanol/water (80:20, v/v) at 200 μL/min for a total run time of 5 min. The column will be operated at room temperature. The retention time of acrylamide and d_3-acrylamide is 1.56 min.

The mass spectrum data will be acquired with positive ion atmospheric pressure ionization (APCI) utilizing the multiple-reaction monitoring (MRM) mode. Transitions for acrylamide and d3-acrylamide were monitored at 72/55 and 75/58, respectively.

Anticipated Outcome:

All the samples processed will be tested for acrylamide levels. The results determine if the addition of amino acids, flavonoids and other polyphenolics affect the formation of acrylamide. With the knowledge obtained from this project, we will be able to make the recommendation on how to best modify the current commercial processing procedures to reduce the level of acrylamide in canned black ripe olives.

Select References:

Acar,O.C., Gokmen, V. Investigation of acrylamide formation on bakery products using a crust-like model. *Molecular Nutrition $ Food Research*, **2009**, 53, 1521-1525

Casado, F. J.; Montaño, A. Influence of processing conditions on acrylamide content in black ripe olives. *Journal of Agricultural and Food Chemistry* **2008**, *56*, 2021-2027.

Casado, F. J.; Montaño, A.; Spitzner, D.; Carle, R. Investigations into acrylamide precursors in sterilized table olives: Evidence of a peptic fraction being responsible for acrylamide formation. *Food Chemistry* **2013**, *141*, 1158-1165.

Charoenprasert, S., Mitchell, A. E. The influence of California-style black ripe olive processing on the formation of acrylamide. *Journal of Agriculture and Food Chemistry* **2014**, 62(34), 8716-8721.

Cheng, J., Chen, X., Zhao, S., Zhang, Y. Antioxidant-capacity-based models for the prediction of acrylamide reduction by flavonoids. *Food Chemistry* **2015**, 168, 90-99.

Cheng, K. W., Zeng, X., Tang, Y. S., Wu, J. J., Liu, Z., Sze, K. H., Chu, I. K., Chen, F., Wang, M. Inhibitory mechanism of naringenin against carcinogenic acrylamide formation and nonenzymatic browning in Maillard model reactions. *Chemical Research in Toxicology* **2009**, 22, 1483-1489.

Claeys, W. L.; De Vleeschouwer, K.; Hendrickx, M.E. Effect of amino acids on acrylamide formation and elimination kinetics. *Biotechnology Progress*. **2005**, 21, 1525-1530.

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International Agency for Research on Cancer. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; World Health Organization (WHO): **1994**; p 389. It is available on the website http://monographs.iarc.fr/ENG/Monographs/vol60/volume60.pdf.

National Toxicology Program. *Toxicology and carcinogenesis studies of acrylamide*; U.S. Department of Health and Human Services: **2012**. It is available on the website http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr575_508.pdf.

Oral, R.A., Dogan, M., Sarioglu, K. Effects of certain polyphenols and extracts on furans and acrylamide formation in model system, and total furans during storage. *Food Chemistry*, **2014**, 142, 423-429.

Sánchez, A. H., Beato, V. M, López-López A., Montaño A. Comparative study of the use of sarcosine, proline and glycine as acrylamide inhibitors in ripe olive processing, *Food Additives & Contaminants: Part A*, **2014**, 31(2), 242–249.

Stumbo, C. R., Purohit, K. S., Ramakrishnan T. V. Thermal process lethality guide for low-acid foods in metal containers. *Journal of Food Science* **1975**, 40(6): 1316–1323.

BUDGET REQUEST (Selina Wang)

Budget year: 2018-2019

Personnel (salaries and benefits): $27,500

- Graduate student GSR- 50%@$55,000

Supplies (instrumental and chemical supplies): $20,000

Travel: $500

- Sample collections and meetings with the processors

Subtotal: $48,000

University overhead 11%: $5,280

TOTAL BUDGET REQUEST: $53,280

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: Food Science and Technology

Project Year: 2018-2019 Anticipated Duration of Project: March 2018 – July 2019

Project Title: Differentiation of olive cultivars using DNA and NMR-based fingerprinting methods

Project Leader:

Dr. Selina Wang

University of California, Davis
Olive Center
Food Science & Tech
Phone: 530-219-1267
E-mail: scwang@ucdavis.edu

Cooperator:
Dr. Emmanuel Hatzakis (Ohio State University)

Commodity: olives

Year Initiated: 2018 Anticipated Duration of Project: March 2018 – July 2019

Problem and Its Significance:

According to the Food and Agriculture Organization (FAO) germplasm database, over 1000 cultivars of olives exist worldwide. Cultivar significantly influences the physical appearance and oil content of the fruit, as well as chemical parameters like phenolic content, fatty acid profile and volatile profile.[1,2,3] As there are price, quality and sensory differences between olives of different cultivars, it is important for California producers to be able to determine which cultivar they are receiving from importers. Morphological characterization and sensory evaluation are the simplest methods for differentiation.[3,4] However, these techniques are unreliable for California-style olives, as processing can change the shape, texture, color and sensory characteristics of the fruit. An accurate and sensitive method to determine the cultivar of processed olives would greatly benefit the California table olive industry.

Genetic markers can be used to differentiate between cultivars of olives. Previous studies have successfully used techniques that characterize variations in DNA among cultivars for cultivar identification and fingerprinting. One popular technique involves the use of single nucleotide polymorphisms (SNPs), or variations in individual nucleotides that occur at specific positions in a DNA sequence. SNPs are abundant in the genome, stably inherited, and conducive to analysis with next generation high-throughput sequencing methods.[5,6] Capitalizing on previous research that has defined cultivar-specific SNPs, we will develop a SNP array designed to detect genetic differences between olives. We will develop an extraction method to salvage DNA from canned, processed olives and use our SNP array for cultivar identification. The challenge we will need to overcome to use this method is ensuring that enough SNPs are included in our array to identify cultivar in the face of DNA degradation, which is likely to occur during California-style olive processing. This project has both scientific novelty and practical implications; it will establish genomic database of processed ripe table olives for the first time as well as provide a useful tool for the California food industry to identify the cultivar of imported products.

Nuclear magnetic resonance (NMR)-based metabolomic analysis has also emerged as a very promising technique for differentiating food products by cultivar and/or geographical origin. Previous studies have used NMR (^{1}H, ^{13}C, ^{31}P)to classify olive oils based on geographical origin.[7–9] A metabolomics fingerprinting approach is used, meaning that resonances are not necessarily assigned to specific compounds. Instead, the full spectra are analyzed using multivariate statistical analysis and samples are clustered based on geographical origin. A sufficient number of samples with known origins are analyzed in order to build a model, which can then be used to predict the geographical origin of an unknown sample. This same approach can be modified to differentiate between cultivars of processed table olives. The composition of olive fruit is more complex than olive oil, meaning that a higher amount of interferences may be present in the spectra. We will test different extraction methods and NMR analyses to determine which method generates the most informative NMR spectra with the greatest sensitivity and resolution. After optimizing extraction and instrument conditions, canned olives of known cultivars will be analyzed and a model for identifying the cultivar of unknown table olives will be developed and validated.

Fatty acid profile (FAP) analysis is a third common method for classifying olives and olive oils based on cultivar. [10,11] The premise is that each cultivar has a distinct ratio of individual fatty acids. Previous studies have successfully used FAP to discriminate between cultivars of California-style olives.[12,13] However, only three common cultivars— Manzanilla, Hojiblanca and Gordal— were analyzed. Results from our previous COC project suggest that FAP can potentially be used to differentiate Mission, Sevillano and Barouni cultivars as well. We will analyze the fatty acid profile of all cultivars relevant to the California industry and, similar to NMR analyses, FAP data will be clustered using multivariate statistics to build a model for identifying unknown samples.

Our *hypothesis* is that olive fruits from different cultivars have distinct metabolic profiles, detectable via NMR spectroscopy, DNA fingerprinting and fatty acid profiles. Integration of

DNA, NMR and FAP analysis will create more robust tools for olive fruit authentication compared to models that rely on individual methods.

Objectives:

To develop DNA and NMR-based fingerprinting methods and fatty acid profiles for differentiating cultivars of processed olives. Color-coded loading plots will be generated from supervised multivariate statistical analysis methods to indicate the significance of specific metabolites in different cultivars and regions.

Experimental Procedures:

Samples: Canned olives of known cultivars (*e.g.* Manzanilla, Hojiblanca, Sevillano, Gordal, Barouni, Mission) will be provided by processors. We will obtain fresh plant material for these cultivars from the USDA National Clonal Germplasm Repository at UC Davis.

Lab supplies: Zymo ZR Plant/Seed DNA MicroPrep kit, ethanol, gloves, pipette tips, PCR supplies for SNP isolation and DNA amplification, Fluidigm EP1 System Genotyping, 5 mm sample tubes, standards, deuterated solvents and instrumental time.

DNA: We will conduct a literature search to identify 192 candidate biallelic SNPs that are variable between cultivars. We will validate these SNPs using DNA extracted from fresh olive fruits. We will design DNA primer sequences that will target these SNPs. Genotyping of DNA will be performed at the UC Davis DNA Technologies Core using the Fluidigm EP1 system. After validation, we will test the performance of these SNPs on identifying cultivar of origin from DNA extracted from canned fruits. If an insufficient quantity of DNA is extracted from canned fruits, we will use a PCR amplification step to amplify the region containing the SNP prior to genotyping. We will aim to produce a 48-SNP panel that can be used as a tool to reliably identify cultivar for industry.

NMR: Method development will be performed by our collaborator Dr. Emmanuel Hatzakis at the Ohio State University (OSU). Polar and nonpolar fractions will be isolated from olive fruit and analyzed using different NMR liquid spectroscopy methods including ^{1}H NMR and ^{13}C NMR analysis. The NMR experiments will be performed in the NMR facility at the OSU using a 700 MHz instrument equipped with a 5mm TXO cryoprobe. Data pre-processing will be performed using AMIX software and principle component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) will be applied to the data using SIMCA-P software. Conditions that provide the most sensitive differentiation will be optimized. We will use the developed method to analyze 30 samples of canned olives from each cultivar and a model will be constructed by PLS-DA. Finally, we will analyze 30 samples of various known cultivars in order to test the prediction ability of the model.

FAP: FAP will be determined following the IOC official method (COI/T.20/Doc. no. 24-2001). Oil will be extracted from fruit using hexane. Methanol/HCl will be used to convert fatty acids into fatty acid methyl esters (FAMEs), which can be analyzed by gas chromatography-flame ionization detection (GC-FID). We will analyze the FAP of 30 samples from each cultivar and, using PLS-DA, construct a model for identifying unknown samples. As with NMR analyses, we will analyze samples of various known cultivars to test the effectiveness of the model.

Anticipated Outcomes:

All the samples will be analyzed by DNA, NMR and fatty acid profiles; the data will be compared to the known cultivars. A genomic and metabolic database of processed ripe table olives will be established for the first time and to be used as a tool for the California industry to identify the cultivar of imported products.

Select References:

(1) Mannina, L.; Dugo, G.; Salvo, F.; Cicero, L.; Ansanelli, G.; Calcagni, C.; Segre, A. Study of the Cultivar–Composition Relationship in Sicilian Olive Oils by GC, NMR, and Statistical Methods. *J. Agric. Food Chem.* **2003**, *51*, 120–127.

(2) Gómez-Rico, A.; Fregapane, G.; Salvador, M. D. Effect of cultivar and ripening on minor components in Spanish olive fruits and their corresponding virgin olive oils. *Food Res. Int.* **2008**, *41* (4), 433–440.

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(4) Rotondi, A.; Magli, M.; Ricciolini, C.; Baldoni, L. Morphological and molecular analyses for the characterization of a group of Italian olive cultivars. *Euphytica* **2003**, *132*, 129–137.

(5) Xanthopoulou, A.; Ganopoulos, I.; Bosmali, I.; Tsaftaris, A.; Madesis, P. DNA fingerprinting as a novel tool for olive and olive oil authentication, traceability, and detection of functional compounds. In *Olives and Olive Oil as Functional Foods: Bioactivity, Chemistry and Processing*; 2017; pp 587–601.

(6) Bracci, T.; Busconi, M.; Fogher, C.; Sebastiani, L. Molecular studies in olive (Olea europaea L.): overview on DNA markers applications and recent advances in genome analysis. *Plant Cell Rep.* **2011**, *30*, 449–462.

(7) Rezzi, S.; Axelson, D. E.; Reniero, F.; Mariani, C.; Guillou, C. Classification of olive oils using high throughput flow 1 H NMR fingerprinting with principal component analysis , linear discriminant analysis and probabilistic neural networks. *Anal. Chim. Acta* **2005**, *552*, 13–24.

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(9) Binetti, G.; Del Coco, L.; Ragone, R.; Zelasco, S.; Perri, E.; Montemurro, C.; Valentini, R.; Naso, D.; Fanizzi, F. P.; Schena, F. P. Cultivar classification of Apulian olive oils: Use of artificial neural networks for comparing NMR, NIR and merceological data. *Food Chem.* **2017**, *219*, 131–138.

(10) Ollivier, D.; Artaud, J.; Pinatel, C.; Durbec, J. P.; Guerere, M. Triacylglycerol and Fatty Acid Compositions of French Virgin Olive Oils . Characterization by Chemometrics. *J. Agric. Food Chem.* **2003**, *51*, 5723–5731.

(11) Giansante, L.; Vincenzo, D. Di; Bianchi, G. Classification of monovarietal Italian olive oils by unsupervised (PCA) and supervised (LDA) chemometrics. *J. Sci. Food Agric.* **2003**, *83*, 905–911.

(12) López-López, A.; Rodríguez-Gómez, F.; Cortés-Delgado, A.; Montano, A.; Garrido-Fernández, A. Influence of ripe table olive processing on oil characteristics and composition as determined by chemometrics. *J. Agric. Food Chem.* **2009**, *57* (19), 8973–8981.

(13) López, A.; Montaño, A.; García, P.; Garrido, A. Fatty acid profile of table olives and its multivariate characterization using unsupervised (PCA) and supervised (DA) chemometrics. *J. Agric. Food Chem.* **2006**, *54* (18), 6747–6753.

BUDGET REQUEST (Selina Wang)

Budget year: 2018-2019

Personnel (salaries and benefits): $41,250

- Graduate student GSR- 50%@$55,000
- Graduate student GSR- 25%@$55,000

Supplies (instrumental and chemical supplies): $19,000

Travel: $500

- Sample collections and meetings with the processors

Subtotal: $60,750

University overhead 11%: $6,683

TOTAL BUDGET REQUEST: $67,433

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department:

Project Year: 2018/2019 Anticipated Duration of Project: 2 years

Project Title: Novel insecticide controls for black scale: general impact and non-target concerns

Project Leaders: *(include names, affiliations, addresses, telephone #s, email)*

Principal Investigator: Houston Wilson
Affiliation: Assistant Cooperative Extension Specialist, UC Riverside
Location: Kearney Agricultural Research and Extension Center (KARE)
Mailing Address: KARE, 9240 S. Riverbend Ave., Parlier, CA 93648
Phone: 559-646-6519 (office) 559-403-9783 (cell)
E-mail: houston.wilson@ucr.edu

Co-PI: Kent Daane
Affiliation: Assistant Cooperative Extension Specialist, UC Berkeley
Location: Kearney Agricultural Research and Extension Center
Mailing Address: KARE, 9240 S. Riverbend Ave., Parlier, CA 93648
Phone: 559-646-6522 (office)
E-mail: kdaane@ucanr.edu

Cooperators: *(include name and affiliations)*
Elizabeth Fichtner, Farm Advisor, Tulare County

Commodity: Olive Relevant AES/CE Project No.:

Year Initiated: 2017 Current Funding Request: $20,108

Problems and Significance:
There are three predominant insect pests in California table and oil olives: black scale (*Saissetia oleae*), olive scale (*Parlatorea oleae*) and olive fruit fly (*Bactrocera oleae*). This proposal directly concerns black scale and indirectly concerns controls for the other olive pests. Over 20 years ago, black scale population dynamics, biological controls, and cultural controls from the northern (e.g., Corning, CA) to southern (e.g., Exeter, CA) table olive range were examined (Daane and Caltagirone 1989, Daane et al. 1991, 2004) and included the description of "open" olive canopies that promoted abiotic (e.g., weather) mortality vs. "closed" canopies that provided better shelter for the scale and reduced abiotic mortality. This work demonstrated that as much as 90% of the scale "crawlers" (first instars) and second instars can die from this abiotic mortality from May to July when temperatures are hot and dry. As such, combinations of mild spring and summer

temperatures, along with closed canopies that reduce temperatures can result in small black scale populations quickly increasing in numbers.

Today, mechanical pruning is being used and perfected in "hedge" style olive production. While this is a benefit to olive management, it may also result in the closed tree canopies that promote black scale outbreaks. The olive industry is still primarily reliant on organophosphates and carbamates for scale control – even though excellent novel materials exist and are used in other crops. Here, we propose to screen some of the more modern insecticides for their effectiveness against black scale as well as evaluate possible non-target effects (e.g., will they disrupt biological control of the olive scale) and synergistic effects (e.g., will they also kill olive scale or olive fruit fly). We will then work to get registration of the best materials for use on olives and develop guidelines (e.g., rates, timing) for their application. Based on conversations with industry cooperators, candidate materials will likely include Esteem (pyriproxyfen), Applaud (buprofezin), Bexar (tolfenpyrad), Sequoia (sulfoxaflor), Sivanto (flupyradifurone), Movento (spirotetramat) and Admire (imidacloprid).

Progress to Date:
This proposal was first discussed in 2015; however, commercial olive fields with appropriate black scale infestations were not found early enough in the spring to conduct the field studies (no funds were requested). We now believe that we will have field sites to conduct this important work.

Objectives:
1) Screen insecticides for their toxicity against black scale (Year 1)

2) Determine non-target or synergistic effects of selected novel insecticides (Year 1)

3) Field test selected insecticides to determine application timing and rate (Year 2)

4) Work with industry and state agencies to extend information on the best commercially available materials (Year 3, no funding required)

Experimental Procedures:

Objective 1 – Lab Trials – Direct and Residual Effects on Black Scale
In Year 1, greenhouse and laboratory studies will be conducted to determine the efficacy of various novel insecticides against black scale. Materials that initially show promise in the direct spray trials will be included in further testing to evaluate residual effects (i.e. how long they last) as well as quantify their specific LD50 and LD90 (lethal dose) against both immature and mature stages (i.e. crawler to adult stages) of black scale.

(1a) Direct Spray Trials Using Potted Oleander
These trials will be conducted using individual potted oleander infested with black scale. Each potted oleander will be inoculated at two different periods to create a scale population with both immature (crawlers and second instar scale) and mature (third instar to adult scale) stages. On each potted oleander, scale will be counted on 10 leaves (for immature stages) and on a 10 cm base

section of the stem (for mature stages). The scale will then be exposed to direct spray of the selected insecticides at manufacturer recommended field rate using a hand sprayer in the greenhouse. Scale survivorship will then be observed at 1, 3, 7 and 14 days after the insecticide application.

(1b) Residual Spray Trials Using Potted Oleander

The best materials from the direct spray trials will be selected for residual spray trials. Using clean oleander plants, selected insecticides will be applied using a hand sprayer in the greenhouse. Following the spray, 10-20 first instar black scale crawlers will be placed on three randomly selected leaves of each treated tree and isolated using clip cages. Inoculations will occur at 1, 3, 5, 7 and 14 days after application. The crawlers will be allowed to settle and feed and then the number of surviving black scale will be determined 14 days after each inoculation. Only first instar crawlers will be used in this experiment as this is the dispersal stage and therefore most likely to encounter a pesticide residue as they colonize trees.

(1c) Determine LD50 and LD90 against black scale life stages

The best materials from the direct spray trials will also be more closely evaluated to determine LD50 and LD90 against various life stages of black scale (i.e. 1st instar crawlers, 2nd-3rd instars, and adult stages) using a Petri dish bioassay technique (Prabhaker et al. 2007). Agar beds will be layered in the base of a 9 mm Petri dish and then 2 cm discs will be cut from freshly collected oleander leaves and placed into the dish. Leaf discs will then be inoculated with 10-20 individual black scale. For contact materials, a Potter spray tower will be used to spray the leaf discs with a series of different concentrations of selected pesticides. Scale mortality will be evaluated 24 hours after the spray. For systemic materials, leaf discs will be punched from potted oleander plants that were sprayed 48 hours earlier.

Experimental Design and Data Analysis

All three experiments will use distilled-water as a control and methidathion (Supracide) will be included as the insecticide standard. Single potted oleander trees or individual Petri dishes will serve as the experimental unit and all insecticide treatments and arthropod-treatment combinations will be replicated 3 to 5 times (depending on the number of materials tested) in a replicated complete block design. Count data from these three trials will be corrected according to Abbot's formula to account for naturally occurring mortality. Generalized linear mixed models will be used to analyze the data in (1a) and (1b), while a probit model and log-concentration probability regressions will be used to analyze data from (1c). All analyses will be carried out using the R statistical computing program.

<u>Objective 2 – Lab Trials – Direct and Residual Effects on Non-Target Organisms</u>

Using the best performing materials from Objective 1, non-target studies will be conducted with residual contact tests against natural enemies (e.g., green lacewings, lady beetles, key parasitoids) and non-target pest species (olive scale and olive fruit fly). Adult and/or larval natural enemy and pest species will be either collected from the cultures in our laboratory or purchased from commercial insectaries.

(2a) Residual Effects on Natural Enemies and Non-Target Pests

Responses of each of the natural enemies and pests to the selected pesticides will be determined using the Petri dish bioassay technique outlined above. Leaf discs (2 cm) will be cut from freshly

collected oleander leaves and then, for contact materials, dipped into each pesticide treatment solution for 30 seconds and allowed to dry for one hour before being put into an agar lined Petri dish. For systemic materials, leaf discs will be punched from potted oleander bushes sprayed 48 hours prior. The appropriate adult or larval stage(s) of the natural enemy / pest species tested will then be placed onto the leaf discs in the Petri dishes. Dishes for the parasitoids will be provisioned with honey streaks as a food source while predator Petri dishes will have the appropriate prey species. Observations of arthropods will be recorded in 24-hour intervals over the course of 4 days and notes will be made on mortality and any noticeable secondary effects.

(2b) Sublethal Effects on Natural Enemies
For materials which do not result in mortality of the natural enemy, sublethal effects will be evaluated by a second experiment where female natural enemies are set up in Petri dishes as previously described and provisioned with either prey or their hosts. Longevity/survivorship, fecundity, sex ratio of offspring and number of prey consumed will be measured.

Experimental Design and Data Analysis
In both experiments, distilled water will be used as a control and individual Petri dishes will serve as the experimental unit within a replicated complete block design with at least 10 replicates with 5 individuals per replicate of each arthropod-treatment combination. As before, count data will be corrected according to Abbot's formula and generalized linear mixed models will be used to analyze the data in the R statistical computing program.

Objective 3 – Year 2 – Field Trials
In Year 2, selected materials from the lab studies will be field tested, primarily to document their effectiveness and determine appropriate application timing based on a biofix of crawler emergence (e.g., spring to summer) or post-harvest applications (e.g., fall to winter). This objective will be fully developed based on the results of the lab trials in Year 1, which will indicate which materials should be tested. We anticipate locating an orchard in the Central Valley as well as a coastal site (if funding from the California Olive Committee is available). Materials that have not been registered for use on olives will require crop destruct and will need to be field tested under different conditions, such as experimental plots at the Kearney Agricultural Research and Extension Center.

At each site, individual trees will serve as the replicate experimental unit. All treatments will be applied at their recommended field application rate (as suggested by industry cooperators). Treatments will be applied using a powered backpack sprayer to single tree replicates with a buffer tree in each direction between all treated trees to prevent treatment drift. Each field site will be set up using a randomized complete block design with blocks aligned with orchard rows and seven replicates per treatment. Black scale will be counted prior to insecticide applications and then at periods throughout the season based on a sampling program previously designed (Daane, unpubl. data). Briefly, for each count, five 30 cm terminal branches from each tree will be collected, taken to the laboratory, and the number of scale will be counted. Samples will be taken prior to insecticide application and at four seasonal periods thereafter to measure adult numbers in spring, crawler emergence in early summer, immature survival in fall, and winter population (typically second and third instars) density.

The field trial will be analyzed as a randomized complete block trial using generalized linear mixed models in the R statistical software. Using mean scale counts per sample unit as the key response variable, a nested analysis with block nested within location as random effects and treatment as the fixed effect will be used first to determine if the data can be analyzed pooling the two locations. If significant interaction with treatment and location are detected, each location will be analyzed separately as a randomized complete block. Means will be separated using Tukey's HSD with α=0.05.

Additional trials may be designed to test different applications periods within specific seasonal periods (e.g., March, April, and May).

Objective 4 – Year 3 – Product Registration

In Year 2 and Year 3, we will work with industry, UCCE personnel and chemical industries to extend information on the most appropriate materials. The project will provide olive growers with more tools for black scale control.

Anticipated Outcomes:

Findings from these studies will be presented to growers as data become available. Presentations will include talks, posters, slideshows, videos, and/or panel and group discussions at relevant grower/PCA/industry meetings as well as field days and seasonal meetings organized by various commodity boards and commissions. We will also disseminate the findings through the UC cooperative extension website to reach out to the maximum possible number of growers in a more convenient way. The information generated in this project will also be initially published in grower magazines such as the Good Fruit Grower, Western Fruit Growers, and American Fruit Grower using grower-friendly vocabulary. We also plan on publishing UC extension bulletins and semi-technical pamphlets to disseminate our findings to the growers. The information will be shared with other researchers through presentations at various professional meetings such as the regional and national meetings of Entomological Society of America, Biological Control in the Western U.S. and other professional meetings. Finally, information generated in this study will be published in peer-reviewed journals such as Biological Control, Journal of Economic Entomology and Environmental Entomology.

Information generated in this project will provide new information to growers and improve their IPM program by helping them select (when necessary) only the reduced risk insecticides that would be least disruptive to natural enemies and other non-target organisms in olive orchards.

Select References:

Daane, K. M., and Caltagirone, L. E. 1989. Biological control in olive orchards: cultural practices affect control of black scale. *California Agriculture* 43(1): 9-11.

Daane, K. M., Barzman, M. S., Kennett, C. E., and Caltagirone, L. E. 1991. Establishment of *Prococcophagus probus* Annecke and Mynhardt and *Coccophagus rusti* Compere (Hymenoptera: Aphelinidae): Parasitoids of black scale in California. *Pan-Pacific Entomologist* 67: 99-106.

Daane, K. M., R. E. Rice, W. W. Barnett, F. G. Zalom, and M. W. Johnson. 2004. Arthropod pests, pp 105-114. *In* G. S. Sibbett and L. Ferguson, [eds.]. *Olive Production Manual.* University of California Agriculture and Natural Resources Publication 3353.

Prabhaker, N., Henneberry, T. J. Toscano, N. C., Naranjo, S. E., Morse, J. G., and Castle, S. J. 2007. Toxicity of seven foliar insecticides to four insect parasitoids attacking citrus and cotton pests. *Journal of Economic Entomology* 100: 1053-1061.

BUDGET REQUEST: Houston Wilson
Budget Year: 2018/2019 Funding Source: COC

	Year 1 (2018)	Year 2 (2019)
Labor	**15,615**	**15,928**
Salary: Laboratory Assistant I Step I ($15.64/hour) @ 100% FTE Apr. 1 – Sept. 30 with 2% increase in Year 2	15,014	15,315
Benefits: Laboratory Assistant I (4%)	601	613
Sub 1 (Labor)	**15,615**	**15,928**
Supplies, Equipment	**1,500**	**1,500**
Supplies: Insect cages, hand sprayers, backpack sprayer, petri dishes, agar, forceps, paint brushes, flagging tape, plastic bags,	1,500	1,500
Equipment:	0	0
Individual Contractors:	0	0
Sub 2 (Labor + Supplies/Equipment)	**17,115**	**17,428**
Travel	**1,000**	**1,000**
Vehicle Use: Car rental, gas, mileage	1,000	1,000
Meeting Attendance:		
Sub 3 (Labor + Supplies/Equipment + Travel)	**18,115**	**18,428**
UCR Overhead @ 11% (0.11 * Sub 3)	**1,993**	**2,027**
Sub 4 (Total to Primary PI)	**20,108**	**20,454**
Subcontracts	**0**	**0**
TOTAL BUDGET REQUEST	**20,108**	**20,454**

PRIMARY PI SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

S. Hunt Walker 11/1/17

Originator's Signature Date

Richard A Redak

Digitally signed by Richard A. Redak
DN: cn=Richard A. Redak, o=University of California, Riverside, ou=Dept. Entomology, email=richard.redak@ucr.edu, c=US
Date: 2017.11.01 14:33:43 -07'00'

Department Chair/County Director Date

Liaison Officer Date

SUBCONTRACT BUDGET REQUEST: (*Secondary PI Name Here*)
Budget Year:_____ Funding Source: COC

Labor:	**(Line 1)**	**$A +$B**
Salary *(describe position(s), percentage FTE or # hours, and hourly rate)*		$A
Benefits (*rate(s)*)		$B
Sub 1		**Line 1 total**

Supplies, Equipment:	**(Line 2)**	**$C+$D+$E**
Supplies: *(be specific. Examples include tape, tags, buckets, traps, safety, chemicals, etc)*		$C
Equipment: (*be specific. Examples include balances, meters, devices, etc*)		$D
Individual contractors: *(be specific: Examples include harvest contractor; fees to RECs, etc*)		$E
Sub 2		**(Line 1+Line 2)**

Travel:	**(Line 3)**	**$F+$G**
Vehicle Use: (*be specific. Examples include mileage for personal vehicle use, fees for motor pool vehicles, travel destination*		$F
Meeting attendance: (*be specific. anticipated travel to meetings such as COC meetings, professional society meetings*)		$G
Sub 3	**(Line 4)**	**(Line 1+Line 2+Line 3)**
UCD/ANR/UCR Overhead @ XX%	**(Line 5)**	**0.XX * Line 4**
Sub 4 (Total Subcontract)	**(Line 6)**	**Line 5 + Line 4**

SUBCONTRACT SIGNATURE PAGE: UNIVERSITY OF CALIFORNIA

_____ _____
Originator's Signature Date

_____ _____
Department Chair/County Director Date

_____ _____
Liaison Officer Date

CALIFORNIA OLIVE COMMITTEE

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Workgroup/Department: UCCE Glenn County

Project Year: 2018 Anticipated Period of Performance: 1 year

Project Title: New materials for Olive Fruit Fly

Project Leaders: Dani Lightle, UCCE Glenn Co., 530-865-1153, dmlightle@ucanr.edu

Cooperators: Bob VanSteenwyk, UC Berkeley, bobvanst@berkeley.edu; Emily Symmes, UCCE Butte County, ejsymmes@ucanr.edu

Commodity: Olive Relevant AES/CE Project No.:

Year Initiated: 2017

Problem and Progress to Date: The olive industry needs new materials for olive fruit fly. Last year we ran two small plot field trials: a full cover application evaluating Assail, Sivanto, and Venerate; and low volume 10gal/ac applications evaluating Venerate, Grandevo & Danitol. Because of the hot weather, fruit fly pressure in 2017 was lower than in recent years and we had little to no damage in any of the low volume applications, including the controls. However, in the full cover trial, we received promising results with Sivanto and Venerate.

Objectives: Continued evaluation of full cover applications of Sivanto and Venerate for olive fly control.

Experimental Procedures: To date (November 1st), we have only just finished collecting data from the 2017 trials. We will determine our experimental approach for next year after fully evaluating the results from this year. Please accept this one- page proposal as our intention to continue this work next year. If invited by the COC, we will submit a detailed proposal in early 2018.

Anticipated Outcomes: Effective options for olive fruit fly control.

Budget: Our budget for 2017 was $19,647. Our budget request for 2018 is unlikely to exceed $25,000.

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project year: 2018 Anticipated Duration of the project: April –November 2018

Project Leader: Jim Stewart Location: Tulare County
Mailing Address: PO Box 1095, Exeter CA 93221
Phone: (559) 730-6243 FAX: (559) 592-4105 E-mail: jsagipmc@verizon.net

Project Title: Southern San Joaquin Valley Olive Fruit Fly Monitoring Project

Cooperating personnel: Bert Quezada, Doug Bigham, Laura Doskocil

Keywords: Olive Fruit Fly, Monitoring, Traps,
Commodity: Olive

PROBLEM AND ITS SIGNIFICANCE:

The monitoring of Olive Fruit Fly (OLFF) in commercial olive groves in the Southern San Joaquin Valley started in 2001. OLFF is potentially the most significant insect pest in commercial Olive.

OBJECTIVES:

The objective of this project would be to continue the monitoring program of adult OLFF in commercial olive groves in the Southern San Joaquin Valley. Detection and seasonal monitoring of OLFF and the accurate timing of control measures, primarily bait sprays, would be the goal of this project. Correlation of fly collections with fruit susceptibility to infestation would indicate to growers when initial bait treatments should be applied. In addition, monitoring would continue to give growers information on the general OLFF population. This information would specific for only the groves being monitored and would be available to growers to aid in making OLFF management decisions in their respective groves in the area being trapped.

PLANS AND PROCEEDURES:

Seven of the nine sites used in the years 2013 to 2017 in commercial olive groves will be set up with traps in April of 2017. One of sites in Strathmore was moved to a nearby grove in 2016 and the South Exeter location will be relocated to a site across the street in 2018. The locations will be Ivanhoe, Woodlake, Exeter, South Exeter, Tonyville, West Lindsay, Strathmore, Porterville and Terra Bella. In addition, a site in the city of Visalia would also be monitored. All of these sites are in Tulare County where a high percentage of the commercial olives are located in the Southern San Joaquin Valley. Many of the sites have been monitored starting in 2001. All traps will be in place by the first week of April and the program will end the last week of October. Two yellow panel traps with ammonium carbonate bait and male pheromone will be used per site. Traps will be serviced and OLFF counted weekly. Reports detailing the number of OLFF found at each location will be submitted to the California Olive Committee and interested parties within 24 hours on a weekly basis during the project.

BUDGET REQUEST

Budget year: April 1, 2018-December 1, 2018

Funding Source: California Olive Committee
Crop Protection Service, Inc.
Ag IPM Consultants, Inc.

Salaries and benefits:		$15,600.00
Supplies:		
Traps, bait and pheromone		1,200.00
Travel:		
Mileage to trap sites		2,400.00
Equipment:		0.00
	TOTAL	$19,200.00

Funding would be split equally between the above listed funding sources.

Total funding from the California Olive Committee would be: $6,400.00

James R. Stewart
Project leader
Ag IPM Consultants, Inc
PO Box 1095, Exeter CA 93221
Phone: (559) 730-6243
Fax: (559) 592-4105

Bert Quezada
Senior Pest Control Advisor
Ag IPM Consultants, Inc
PO Box 1095, Exeter CA 93221
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Fax: (559) 592-4105

Ern's Pest Control

Project Plan/ Research Grant Proposal

Project Year: 2018

Project Leader: Ernie Simpson
Mailing Address: 320 County Road 15 Orland, California 95963
Phone: 530-865-9829 Cell: 530-518-4685
E-mail: ernsimp17@sbcglobal.net

Cooperator: Dani Lightle, Orchards Advisor, UC Cooperative Extension, Orland

Commodity: Olive

Problem and its Significance:

Since the detection of Olive Fruit Fly in California in 1998, it has been a concern to olive growers in commercial orchards; preventative sprays are necessary. Trapping to monitor the Olive Fruit Fly populations in individual orchards is recommended. This will allow growers and PCA's to follow trends to their orchards and help evaluate spray program efficacy. Having an idea of area-wide population trends will help growers and PCA's interpret the results from their orchards.

Objectives:

1: Provide timely information to area growers regarding area-wide olive fruit fly population trends.
2: Continue to develop a historical perspective of olive fruit fly populations for the area.

Plans and Procedures:

Starting in early April plastic McPhail traps using Torula yeast tablets dissolved in water as the bait will be placed in one tree at 12 sites (6 in Glenn County and 6 in Tehama County). The same sites that have been used in previous years will be monitored again to allow for comparison of current years trap catches to previous years. Earlier work in Glenn and Butte Counties has shown that the plastic McPhail traps catch more flies than the commonly used yellow panel trap. Traps will be checked and flies counted weekly. The results and field observations will be posted on the Sacramento Valley Orchards website (www.sacvalleyorchards.com) and reported via email to the COC for further distribution. Trapping results will be reported as male and female flies for individual traps and combined by site. Trapping and reporting will be continued through December or until trap catches decline for the year.

Budget Request

Budget Year: 2018

Funding Source: California Olive Committee

Salaries	$4735
Supplies and Expenses: Trapping Supplies	$ 365
Travel 2545 mi.	$1800
This may vary due to fuel prices	
Total	$6500

Originator's Signature _____

Ernie Simpson

AES/CE MAR 84

Workgroup: Olive
Department: Plant Pathology/UCR

University of California
Division of Agricultural Sciences
PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2018 Anticipated Duration of Project: 2nd of 3 years

Principal Investigators: J. E. Adaskaveg

Cooperating: D. Thompson, H. Förster, and K. Nguyen

Project Title: Epidemiology and management of olive knot caused by *Pseudomonas savastanoi* pv. *savastanoi*

Keywords: Bactericides, copper enhancing compounds, antimicrobial natural products, biological controls

JUSTIFICATION/ BACKGROUND

Olive knot caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* (*Psv*) is a serious disease of olives (*Olea europaea*) worldwide (8). The pathogen enters through wounds causing outgrowths (knots, tumors, galls) on branches and infrequently on leaves and fruit. Olive knot is one of the most economically important diseases of olives as infection may lead to tree defoliation, dieback, and reduced tree vigor, which ultimately lowers fruit yield and quality (6). *Psv* can survive epiphytically on olives but the main sources of inoculum are bacteria living within knots (7). Large quantities of bacterial ooze can be exuded upon wetting knots. This exudate is disseminated by rain, wind, insects, birds, as well as human activity. The opportunistic pathogen takes advantage of wounds caused by natural leaf abscission (4), frost, and hail, as well as cultural practices such as pruning and harvesting. These latter practices also lead to direct mechanical damage of the knots, exposing and spreading inoculum to healthy tissue. After entering its woody host, the pathogen actively induces knot formation through the production of indoleacetic acid (IAA) and cytokinins (2). In California, infections occur mostly during the rainy season (late fall, winter, and spring) but knots do not develop until new growth starts in the spring. Infections can occur at low temperatures (-5° C) and thus, wetness is the main limiting factor for the disease. None of the currently grown olive cultivars is resistant to the pathogen (5).

Control of olive knot is difficult, and growers rely on applications of copper-based bactericides as the only effective foliar treatment. Manual application of cresol- and xylenol-based compounds (Gallex) to knots can eliminate the knot pathogen but is unfeasible on a commercial scale. Copper has been extensively used in olive production for many years for the control of diseases such as peacock spot and olive knot. Reliance on a single active ingredient has led to our detection of copper resistance in *Psv* strains from a commercial olive orchard. The incidence of copper resistance is currently very low, accounting for only 2% of the total strains collected in different olive growing regions of California. When resistant strains were inoculated to Arbequina and Manzanillo olive wounds, application of copper provided reduced or no control as compared to inoculation with a sensitive strain. Copper-resistant strains caused less disease on leaf scars as compared to Cu-sensitive strains, but still resulted in a high incidence of disease over a range of inoculum concentrations. Therefore, there is a potential risk of copper resistance spreading with continued and sole use of copper. This necessitates the development of new bactericides or copper-activity-enhancing materials to overcome resistance. The latter strategy has proven to be effective for walnut blight management where copper resistance in *Xanthomonas arboricola* pv. *juglandis* is common and copper-mancozeb mixtures have provided exceptional control. Mancozeb can no longer be registered on new crops but other copper-enhancing alternatives can be evaluated. Salicylidene benzoylhydrazone (SBH) was recently discovered to display synergism when combined with copper against *Alternaria solani* causing early blight of tomatoes. We performed preliminary tests with a derivative of this molecule with promising results with several genera of phytopathogenic bacteria including *Psv*. Low concentrations of metallic copper combined with SBH were highly inhibitory in vitro against a copper-resistant *Psv* strain while copper or SBH by themselves at the same concentrations were not effective. Field trials in 2017 on managing olive knot, SBH-copper, however, did not improve performance of copper. Other derivatives of SBH will be supplied by Dow AgroSciences, and these will be tested in 2018.

Other potential bactericides have also been made available to us by agrochemical registrants in 2017. These include a nanoparticle zinc product called Zinkicide and experimental inhibitors of type III secretion systems in plant pathogenic bacteria. The latter compounds are novel in their mode of action. They act on the mechanism that delivers bacterial proteins into the host cells that are necessary for *Pseudomonas* species to cause disease. Currently, we are testing Zinkicide and three experimental type III secretion system inhibitors.

We have been instrumental in the development of the new agricultural antibiotic kasugamycin (commercial name Kasumin) for several bacterial diseases of agronomic crops in the United States. Kasugamycin has high activity against *Erwinia* (1) and *Pseudomonas* species and moderate activity against *Xanthomonas* species and other plant pathogenic bacteria. We found it to be the most promising new treatment for preventing olive knot in our extensive field studies, including in a commercial application to inoculated branches. Kasugamycin is currently federally registered on pome fruit crops, whereas use on olives was approved as an "A" priority by inter-regional project 4 (IR-4) for the 2015 season. Kasumin is still in the IR-4 program with the final report and submission to the EPA pending in 2018 with a possible 2019 registration. Kasugamycin would greatly complement current copper sprays and could be used in rotation or mixtures with copper. Oxytetracycline was also submitted to IR-4 and is in the field trial phase of the IR-4 program for establishing tolerances. We will conduct additional studies with oxytetracycline to potentially improve its efficacy by using registrant-recommended adjuvants. New antibiotic registrations, however, find little acceptance with regulatory agencies, and we are currently in discussion with EPA to develop a science-based approach on the use of antibiotics in plant agriculture.

In addition to developing conventional chemical compounds, research on alternative materials such as biopesticides and food additives may provide new modes of action for managing olive knot. Biopesticides such as Serenade contain the gram-positive bacterium *Bacillus subtilis* (strain QST 713) that produces various compounds that are antagonistic against a broad range of fungal and bacterial organisms. In our efficacy trials, Serenade and Serenade-copper mixtures, however, were not effective at recommended rates.

Several food additives that are considered 'generally recognized as safe' (GRAS) have antimicrobial properties. They are often naturally produced molecules of gram-positive *Streptomyces* species. Although these compounds are typically applied to food products as preservatives, they may have potential for controlling plant diseases when applied as a foliar treatment. Integration of these alternative materials with conventional treatments may improve disease control, reduce the risk of resistance development, and provide olive growers with more resources for managing olive knot. In 2017, we evaluated nisin, epsilon-poly-L-lysine, and lactic acid and all showed similar efficacy to copper in reducing olive knot on leaf scars, but not on lateral wounds. This information is still valuable because rotational programs could be developed with different modes of actions for different phases of the disease, i.e., leaf scars or lateral wounds occurring during leaf drop or harvest and pruning, respectively. These materials are registerable for conventional and possibly organic treatments.

RESEARCH OBJECTIVES

1) **Develop new bactericides and potential enhancers of copper activity against *Psv***
   a) In-vitro sensitivity of *Psv* to Zinkicide, Type III secretion system inhibitors, and copper mixtures with new SBH derivatives (using selected ratios).
   b) Efficacy of new bactericides in comparison with kasugamycin for the management of olive knot caused by copper-sensitive and -resistant strains of *Psv* in field studies.
      i) Zinkicide
      ii) Potential enhancers of copper activity - new SBH derivatives.
      iii) Type III secretion system inhibitors
      iv) Oxytetracycline formulations in combination with adjuvants recommended by the registrant.
2) **Evaluate several food additives and a sanitizer for the control of olive knot**
   a) Determine the efficacy of the GRAS food additives nisin, epsilon-poly-L-lysine, and the GRAS sanitizers lactic and citric acid in field studies for the management of olive knot.
3) **Continue to support the registration of the antibiotics kasugamycin and oxytetracycline** - UV blockers and stabilizers and EPA policy.

PLANS AND PROCEDURES

1) Develop new bactericides and potential enhancers of copper activity against *Psv*.

1a. To evaluate the in vitro toxicity of Zinkicide, the spiral gradient endpoint (SGE) method will be used where bacterial strains are exposed to a bactericide concentration gradient on a single agar plate. To evaluate new potential enhancers of copper activity against *Psv*, a dilution plate method will be combined with the SGE method. Agar media will be amended with fixed concentrations of copper. Subsequently, derivatives of SBH will be applied to the plates in radial concentration gradients using a spiral plater. Suspensions of *Psv* strains will be streaked radially onto the amended media. This will allow the determination of minimal inhibitory values for *Psv* at different ratios of copper and SBH derivatives. These data will then be used to calculate appropriate field rates.

1b,c. Zinkicide, copper-SBH mixtures, Type III secretion system inhibitors, and oxytetracycline will be tested in the field on Arbequina and Manzanillo olives at UC Davis. Plants will be wounded with lateral and leaf scar wounds. Lateral wounds on 1-2-year-old twigs will be made using a scalpel by removing the bark and exposing cambial tissue. Leaf scars will be made by pulling leaves off the same twigs. In addition, wounds from natural leaf drop will be used. Treatments will be sprayed onto wounds before inoculation with a suspension of copper-sensitive or -resistant *Psv* strains. SBH derivatives will be applied using rates based on the laboratory tests. Oxytetracycline will be used in combination with recommended adjuvants because it is especially vulnerable to UV-degradation. Treatments will be compared to Kasumin and copper by itself. The efficacy of treatments will be assessed as the percent incidence of knots forming on treated, inoculated wounds as compared to wounds that are treated with water and inoculated (i.e., controls).

2) Evaluate several food additives for the control of olive knot. Field tests will be conducted on Arbequina and Manzanillo olives to evaluate the efficacy of nisin, epsilon-poly-L-lysine, and lactic acid treatments against *Psv*. The same wounding, treatment application, inoculation, and evaluation procedures will be used as described above.

3) Continue to support the registration of the antibiotics kasugamycin and oxytetracycline. An inter-commodity and industry group will continue to work with the Minor Crop Farmer Alliance to recommend an EPA policy change towards the use of antibiotics in plant agriculture. Specifically, a new internal EPA Guidance Document (GD) for use of antibiotics in plant agriculture needs to be developed based on science. Historically, EPA GD 152 for registration of antibiotics in animal husbandry is used for all requests in agriculture. Additionally, we will continue to work with a USDA working group to address CODEX initiatives for establishing policies on all antibiotic use in agriculture including animal and plant uses.

Benefits to the industry

For management of olive knot, in addition to cultural methods, sanitation practices, and the labor-intensive Gallex, only copper materials and the natural product Regalia are currently available. We obtained improved performance of copper when applications were made within 24 h of wounding events (e.g., harvesting, pruning, hail storms, freezing) as compared to later applications, and with high labeled rates of copper. In our previous research we also showed that copper resistance is currently not widespread and is only found locally where copper has been used for many years. Because copper-resistant strains of Psv were found to be virulent and likely competitive, and because they were not genetically clonal, there is a risk of further spread of copper resistance. Therefore, alternatives are needed for a sustainable and effective management program. We initiated the registration of the new agricultural antibiotic kasugamycin that was registered in 2014 on pome fruit, and of oxytetracycline through the IR-4 program. Kasugamycin showed high activity against olive knot especially in mixtures with copper. Mancozeb as a mix partner with copper was considered by us and the industry, but EPA has denied any new registrations. In 2017, we evaluated new copper activity-enhancing compounds that, however, were not effective; therefore, new derivatives of SBH will be evaluated in 2018 in cooperation with a US registrant (Dow AgroSciences). Working with Brandt Corp., we will also test new bactericides such as Zinkicide, a nanoparticle zinc product. Other companies will provide Type III secretion system inhibitors and natural products (nisin, epsilon-poly-L-lysine, and the GRAS sanitizers lactic and citric acid) for evaluation against bacterial plant diseases. The registration of several materials for olive knot management will allow the implementation of anti-resistance strategies and will prevent over-use of any single mode of action bactericide. Still, integrated practices will be critical for the successful management of the disease. Any

bactericide or biological treatment will be most effective when pathogen population levels are at a minimum and the host is less susceptible.

References

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2. Comai, L., and Kosuge, T. 1980. Involvement of plasmid deoxyribonucleic acid in indoleacetic acid synthesis in *Pseudomonas savastanoi*. J. Bacteriol. 143: 950-957.
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4. Hewitt, W. B. 1939. Leaf scar infection in relation to the olive knot disease. Hilgardia 12:41-66.
5. Penyalver, R., García, A., Ferrer, A., Bertolini, E., Quesada, J.M., Salcedo, C.I., Piquer, J., Pérez-Panadés, J., Carbonell, E.A., del Río, C., Caballero, J.M., López, M.M., 2006. Factors affecting *Pseudomonas savastanoi* pv. *savastanoi* plant inoculations and their use for evaluation of olive cultivar susceptibility. Phytopathology 96:313–319.
6. Schroth, M.N., 1973. Quantitative assessment of the effect of the olive knot disease on olive yield and quality. Phytopathology 63:1064.
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8. Young, J.M., 2004. Olive knot and its pathogens. Australasian Plant Pathology 33:33–39. doi:10.1071/AP03074

Budget Request:

Funding Source: California Olive Commission and California Olive Oil Commission

Budget Request with UC indirect costs:

Budget Year: 2018 Funding Source*:	OOC	COC	Total Budget
Salaries and Benefits: Post-Docs/RAs	7,000	7,000	14,000
Lab/Field Ass't	1,000	1,000	2,000
Subtotal	8,000	8,000	16,000
Employees' Benefits**	4,500	4,500	9,000
Subtotal	12,500	12,500	25,000
Supplies and Expenses	0	0	0
University Land and Orchard charges	1,000	1,000	2,000
Operating Expenses/Equipment Travel	0	0	0
Travel	1,500	1,500	3,000
Direct Cost Totals Total	**$15,000**	**$15,000**	**$30,000**
Off Campus IDC @ 11%		1,650	1,650
Total Budget Requested	**$15,000**	**$16,650**	**$31,650**

J.E. Adaskaveg

Originator's Signature (PI) Date: Oct. 31, 2017

Katherine Borkovich

Dept. Chair (Riverside Campus) Date: Oct. 31, 2017

Liaison Officer Date:

*******ACTION REQUIRED*******

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: 2018 RESEARCH PROJECT

RECOMMENDATION: THAT the Subcommittee approve research project for 2018.

BACKGROUND: Each year the Research Subcommittee approves various research projects funded by the Committee. The Subcommittee must which proposed projects to recommend to the Committee for funding. A budget of $328673.75 is purposed based on the submitted projects.

2018 RESEARCH PROPOSALS FOR THE CALIFORNIA OLIVE COMMITTEE

TOPIC	LEADERS	AMOUNT
A new fruit removal head for an olive harvesting system	Reza Ehsani	$45,741
Canopy management, tree hedging and topping to optimize yield	Rich Rosecrance	$31,075
Propagating Dwarfing Olive Rootstocks and Establishing a Long Term Orchard	John Preece Louise Ferguson	$35,442.75
Managing Alternate Bearing in Olive with PGRs and Pruning	Carol Lovatt Elizabeth Fichtner	$20,698
Evaluation of Several Promising Additives for Reducing Acrylamide in Black Ripe Table Olives	Selina Wang	$53,280
Differentiation of olive cultivars using DNA and NMR-based fingerprinting methods	Selina Wang	$67,433
Novel insecticide controls for black scale; general impact and non-target concerns	Houston Wilson Kent Daane	$20,454
New Materials for olive fruit fly	Dani Lightle	$25,000*
Southern San Joaquin Valley Olive Fruit Fly Monitoring Project	Jim Stewart	$6,400
Sacramento Valley Olive Fruit Monitoring Project	Ernie Simpson	$6,500
Epidemiology and management of olive knot caused by *Pseudomonas savastanoi* pv.*savastanoi*	J. E. Adaskaveg	$16,650
Total		$328,673.75
* budget estimate; actual budget pending results of current year's project		

FISCAL IMPACT: $328,673.75

* * * * *INFORMATION * * * * *

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: NO-COST EXTENSIONS

BACKGROUND: Each year, researchers will request a no-cost extension should their program run past the fiscal year. The Committee adopted a policy a few years ago that allows the Executive Director in conjunction with the Chairman to approve requests for no-cost extensions.

*******ACTION REQUIRED*******

FROM: RESEARCH SUBCOMMITTEE

SUBJECT: INTER-ITEM TRANSFERS OF THE RESEARCH BUDGET

RECOMMENDATION: THAT the Committee grant authority to the Executive Director and Chairman for inter-item transfers of the Research Budget.